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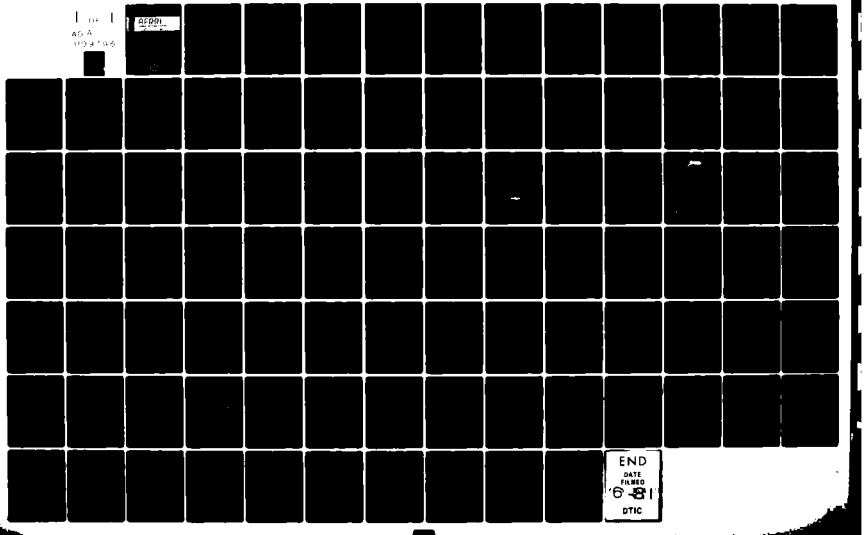

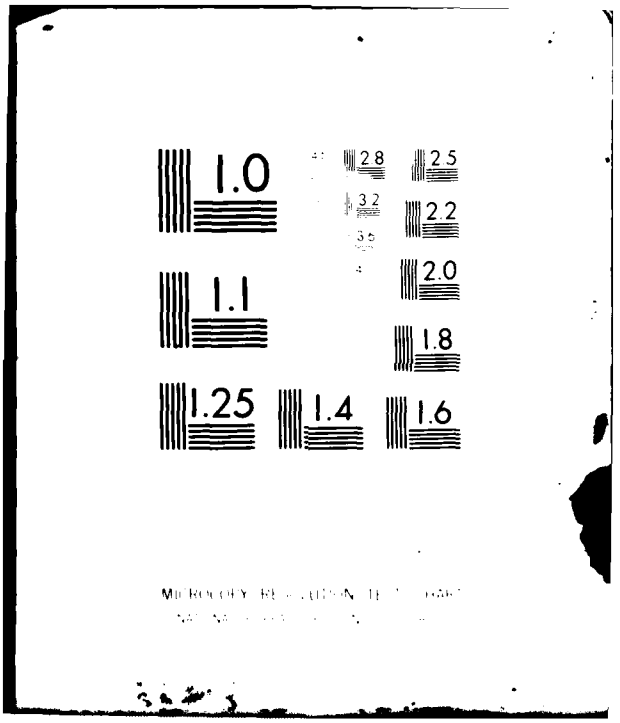
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1 OCTOBER 1977 - 30 SEPTEMBER 1978

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Studies involving human patients were performed in conformity with the "recommendations guiding doctors in clinical research" as stated in the Declaration of Helsinki of the World Health Medical Association (1964).

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This report contains a summary of the research projects of the Armed Forces Radiobiology Research Institute for the period 1 October 1977 to 30 September 1978.

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THESE PROJECTS HAS BEEN CARRIED OUT BY THE FOLLOWING DEPARTMENTS WITHIN THE INSTITUTE =

~~CONTENTS~~

BEHAVIORAL SCIENCES DEPARTMENT

The Behavioral Sciences Department investigates changes in learned behavior in experimental animals. The tasks to which the animals are trained represent activities important in military operational situations. Alterations in learned behavior after exposure to sources of ionizing radiation have been the principal focus of research projects. Also conducted is the evaluation of chemicals from the military working environment for their potential in causing both general systemic pathology and alterations in performance.

Research efforts within the Department are conducted within two Divisions: the Experimental Psychology Division and the Physiological Psychology Division. Efforts are directed toward describing the change in performance and the development of dose-response curves for the agent of interest. Animal models for investigating sites within the central and peripheral nervous systems are used to develop hypotheses of mechanisms of action. These basic studies use methods of experimental neuropharmacology, electrophysiology, biophysics, and biochemistry.

NEUROBEHAVIORAL ANALYSIS OF DRUG RADIATION EFFECTS

Principal Investigators: H. Teitelbaum and G. A. Mickley
Collaborators: W. L. McFarland, P. Giammatteo, and P. Sekre
Technical Assistance: J. F. Lee and B. A. Dennison

Behavior techniques in the rat have been used to study sensitivity of the lateral hypothalamic system to supralethal whole-body doses of high-energy electrons.

After exposure to 10 krad of high-energy electrons, rats experience an early transient incapacitation (Figure 1) characterized by symptoms similar to those associated with lateral hypothalamic lesions: namely, akinesia and decrements in various motivated behaviors (Figure 2). Experimenter-imposed electrical stimulation of the lateral hypothalamus in akinetic, irradiated animals produced a locomotor response similar to that seen in nonexposed rats (1). Furthermore, when allowed to self-stimulate, the irradiated subjects persisted in vigorous self-stimulation of the lateral hypothalamus (Figures 3, 4) whereas self-stimulation rates of other subcortical sites showed marked attenuation (Figure 5). The lateral hypothalamus is apparently less sensitive to ionizing radiation than are the other subcortical structures sampled.

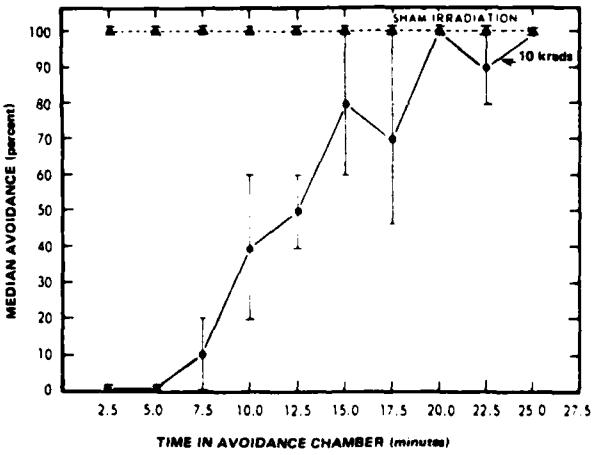

Figure 1. Ten thousand rads of high-energy electrons produce an early transient incapacitation in avoidance responding. Subjects return to normal 100% performance 20-25 min after exposure. Variance measures are represented by quartile range.

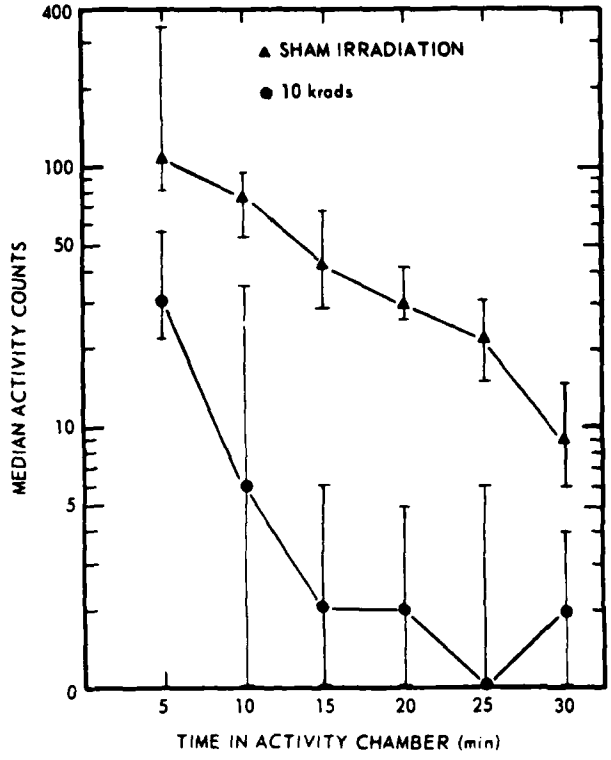

Figure 2. Ten thousand rads of high-energy electrons cause spontaneous locomotor activity to drop drastically (note logarithmic scale on ordinate). Variance measures are represented by quartile range.

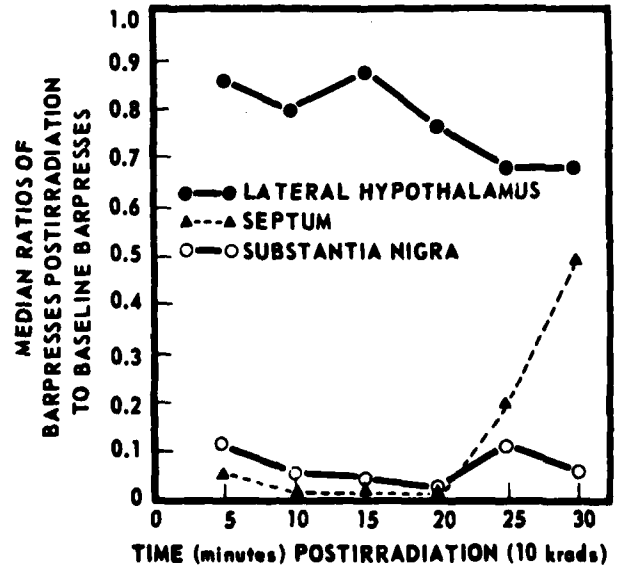

Figure 3. Vigorous self-stimulation of lateral hypothalamus persists after radiation exposure even though similar behaviors mediated by other subcortical structures are strongly attenuated.

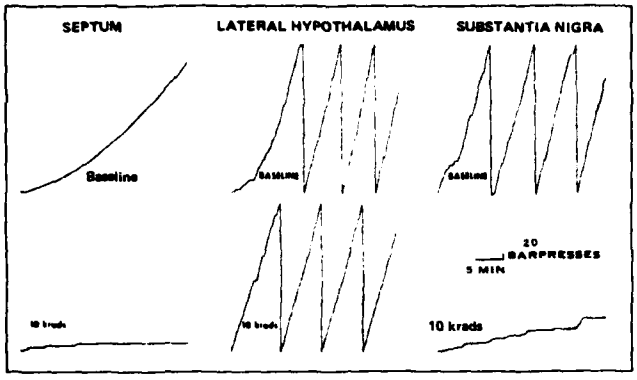

Figure 4. Cumulative records of bar presses for brain stimulation at different sites before and after radiation exposure. Compared to other subcortical sites, lateral hypothalamic self-stimulation is relatively insensitive to radiogenic alteration.

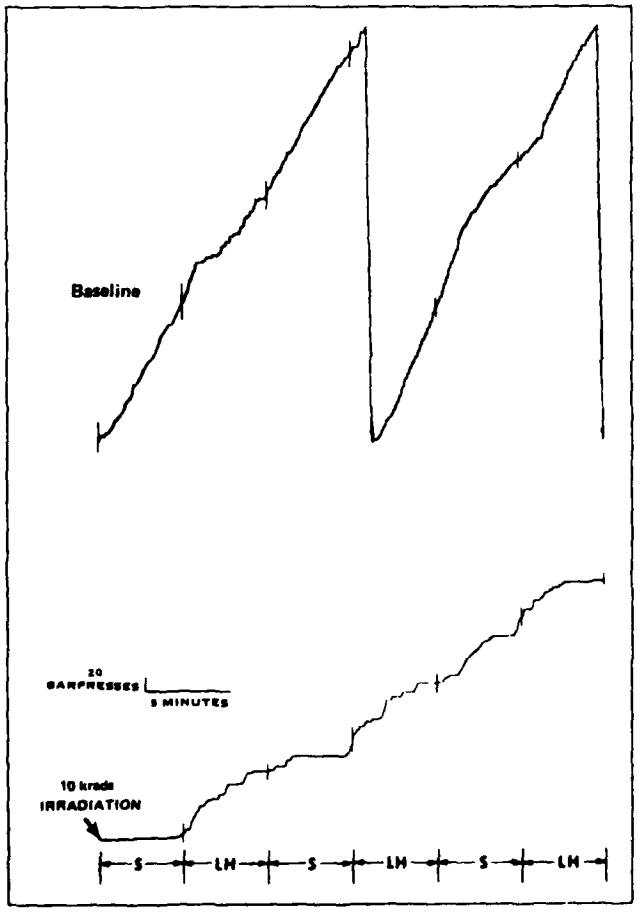

Figure 5. Cumulative records of animal with electrodes in both lateral hypothalamus (LH) and septum (S) indicate that it will continue to press a bar post-irradiation to receive stimulation of LH but not S. Some recovery at both sites is seen after 20 min.

REFERENCE

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DISORDERS OF MOVEMENT AFTER RADIATION EXPOSURE

Principal Investigators: H. Teitelbaum and G. A. Mickley
Collaborators: W. L. McFarland, P. Giammatteo, and P. Sekre
Technical Assistance: J. F. Lee and B. A. Dennison

Goals of this research effort have been to investigate the radiation-induced incapacitation that begins minutes after irradiation--the early transient incapacitation phenomena (ETI)--through the use of pharmacological agents that mimic the ETI. By inducing catalepsy with both radiation and chemicals, the effects can be compared. The chosen drugs have known sites and mechanisms of action within the central nervous system. When such agents successfully mimic aspects of radiation injury, data are provided that allow radiation research efforts to efficiently explore similar mechanisms of action (1).

The recent study of haloperidol catalepsy has directed attention to the lateral hypothalamus and reticular formation. Also it has been demonstrated that electrical stimulation of these areas abolishes the pharmacologically induced catalepsy.

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PERFORMANCE OF PHYSICALLY CONDITIONED RHESUS MONKEYS FOLLOWING MIXED GAMMA NEUTRON IRRADIATION

Principal Investigator: C. G. Franz
Technical Assistance: L. Clark and J. R. Harrison

The dose-response curve for physically conditioned animals exposed to ionizing radiation is being constructed. This information is being assembled for the U.S. Army to incorporate into documents related to operational field problems.

A nonmotorized physical activity wheel was developed and fabricated, and 40 rhesus monkeys were physically conditioned through its use. The animals were trained to move at a rate of not more than 5 revolutions per minute (rpm) and not less than 1 rpm until able to sustain a work/rest schedule of 10 min work/5 min rest for a total of 6 hours. Training time for the physical activity wheel was about 6 months.

After baseline determinations, the animals were exposed to a pulse delivered by the AFRRI TRIGA Reactor configured to give a neutron-to-gamma ratio of 3.0. The range of doses was from 4800 to 1180 rads. Analysis of preliminary data indicates a median effective dose of 1825 rads.

PHYSIOLOGICAL AND BEHAVIORAL EFFECTS OF PETROLEUM-DERIVED AND SHALE-DERIVED JP5 ON RATS

Principal Investigators: V. Bogo and R. W. Young, *AFRRI*
Collaborator: T. Hill, *Naval Medical Research Institute*
Technical Assistance: G. G. Kessell

The U.S. Navy is assessing alternatives to petroleum-derived jet propulsion fuel number 5 (JP5) through study of the feasibility of extracting and refining JP5 from shale. Since no baseline data exist, the relative toxicity of petroleum-derived and shale-derived JP5 should be determined as an integral part of this feasibility study.

Three studies were conducted to set upper limits for the toxicity of petroleum JP5. They concentrated on survival, necropsy, and histopathological findings of Long-Evans and Sprague-Dawley male rats exposed to JP5 per os in multiple and single doses of 5-60 ml/kg. In addition, observations were made on general condition, spontaneous behavior, home-cage activity, food intake, water consumption, and weight. The $LD_{50/14}$ (lethal dose in 50% of animals after 14 days) for JP5 could not be determined, since no deaths occurred within 14 days of treatment with doses of up to 5% of the animal's body weight (48 ml/kg) and since only 33% of the animals died at a dose of 6% of body weight (Table 1). Necropsy and histopathology findings for the subjects that completed the studies were unremarkable. This was probably related to the 14-day interval between dosing and data acquisition, since recovery was possible. (Follow-up studies, in which the subjects will be serially sacrificed after dosing, are being conducted specifically to gather this information.) Instances of hair discoloration (a yellow, soiled appearance) as well as oiliness and matting were noted from days 1-4. These hair manifestations progressed upward and forward from the genital area in a dose-response manner for extent and duration of effect. Alopecia, erythema, and skin lesions were seen in some of the higher dose subjects, starting on day 4.

Table 1. Acute JP5 Toxicity for Orally Dosed Sprague-Dawley Rats

Dose Group (mg/kg)	% of Body Weight	Number Subjects	Deaths (14 Days)		
			Petroleum	Shale	
			Hess, St. Croix	Gary Western	Exxon
60	6	6	2	6	5
48	5	6	0	6	0
38	4	6	0	6	0
30	3	6	0	5	0
24	2	6	0	2	0

Signs of neurobehavioral involvement were also observed. Specifically, home-cage, overnight activity of all JP5 dose groups demonstrated a significant transitory, one-day increase on the day after exposure, followed by a depression in activity (Figure 1). Additionally, the petroleum-dosed animals were observed to be aggressive after dosing. Several cases of self-mutilation were observed at levels of 8-30 ml/kg, and frequent instances of hypersensitivity to touch were noted. Specific function testing of the transitory increase in activity and hypersensitivity was done in a later study. However, the accelerod test of motor integration and the shock titration test of peripheral sensitivity did not produce significant differences between the control animals and the treated animals when tested the day after dosing.

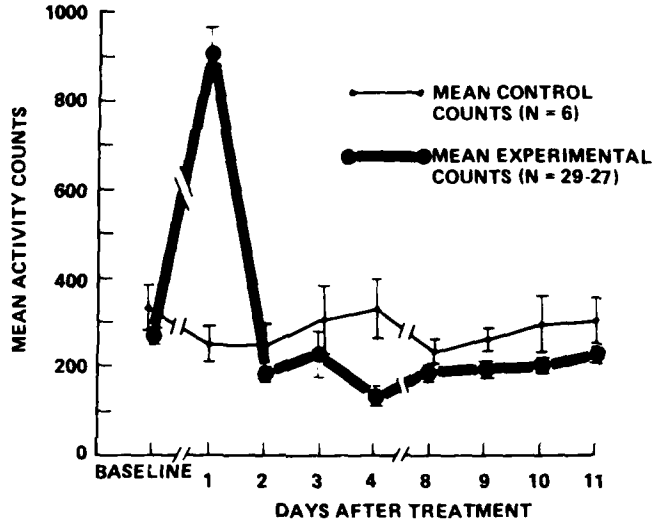

Figure 1. Mean (SE_m) spontaneous activity for Sprague-Dawley rats orally dosed with petroleum-derived JP5

In addition to the petroleum-derived JP5 testing, preliminary comparisons have been made between petroleum-derived and shale oil-derived JP5 (Gary Western and Exxon refining processes). The shale-derived JP5 from the Gary Western process was by far the most toxic (Table 1). The $LD_{50/14}$ for the Gary Western JP5 was 26 ml/kg whereas the petroleum and Exxon shale products did not produce deaths within 14 days, until the animals were dosed at greater than 5% of body weight. Chemical characterization analyses suggest that the lower $LD_{50/14}$ of the Gary Western shale fuel may be due to its nitrogen content, which is higher than that of the other two JP5 products.

During the next fiscal year, two types of studies will be conducted: (a) Further specific function testing to elaborate on the behavioral findings of the oral work. (b) Inhalation studies to simulate a more meaningful route of occupational exposure. A laboratory for the inhalation studies will be assembled with the expectation that the collaborator from the Naval Medical Research Institute, LCDR Hill, will join the primary research team.

ALTERATIONS IN STRIATAL DOPAMINE RELEASE AFTER ELECTRON IRRADIATION COMPARED TO ETHANOL EFFECTS

Principal Investigator: W. A. Hunt
Technical Assistance: T. K. Dalton

The complexity of radiation effects lends itself to companion experiments wherein radiation effects are compared to effects of an agent of some known mechanisms and known sites of action.

In an attempt to assess dopaminergic activity, we investigated the measurement of striatal dopamine release in vitro in the rat as a function of radiation after ethanol insult (1).

A single 10,000-rad dose of high-energy electrons was shown to induce an increase in dopaminergic activity and cholinergic activity in the caudate nucleus of the rat brain as assessed by potassium-stimulated dopamine release in vitro and by high-affinity choline uptake (2). These alterations occur during early transient incapacitation (ETI) and dissipate as the animal recovers behaviorally, within about 30 min after irradiation. Although the responses observed resemble those resulting from blockade of dopamine receptors, no radiation-induced changes were found in dopamine-sensitive adenylate cyclase activity and in tritium-haloperidol binding, two indices of dopaminergic receptor function. The data suggest that changes in dopaminergic and cholinergic activity are associated with the development of ETI and that those changes may play a role in the behavioral decrement observed during this condition.

Acute ethanol administration demonstrated accelerated striatal choline uptake but a reduced hippocampal choline uptake in a dose-dependent manner (3). These responses were reversible after complete elimination of ethanol (4). Elevation of striatal choline uptake in ethanol-dependent animals lasts at least 3 days after withdrawal.

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BIOCHEMISTRY DEPARTMENT

The Biochemistry Department is concerned with elucidating the mechanisms of damage, protection, and repair in biologic systems exposed to ionizing radiation, nonionizing radiation, and toxic chemicals. Of major interest are studies of (a) the effects of radiation and toxic chemicals on cell macromolecular constituents and membrane structure and function and (b) the development of biochemical indicators of radiation injury.

The Department comprises three divisions:

The Physiological Chemistry Division focuses on the development of biochemical markers of early and late effects of radiation exposure. Specific projects include determination of radiation-induced changes in serum glycoproteins and trace metals, modulation of effects of partial- and whole-body irradiation by immunopharmacologic means, and determination of radiation-induced changes on cellular membrane sterols and other lipid components. Collaborative studies with the National Cancer Institute and other medical centers include research on the immunological effects of radiation. The nature of radiation-induced lipid peroxidation and radioprotection by chemical adjuvants are also being investigated.

Research objectives of the Molecular Biology Division are (a) determination of the effects of ionizing radiation, nonionizing radiation, and chemical agents on the mammalian central nervous system, and (b) elucidation of the mechanisms underlying these changes. This includes studies on radiation-induced and chemical agent-induced changes in the levels of neurotransmitters and enzymes involved in their metabolism and in the blood-brain barrier, and also studies on the effects of radiation on lysosomal structure and function, with emphasis on adenyl-guanyl cyclase and prostaglandin metabolism. In addition, the effects of low-level radiation on the biochemically developing brain (newborn animals) are being investigated. Emphasis is on isolation and identification of a humoral factor (circulating myodynamic agent) that seems to be responsible for the observed cardiac failure after exposure to ionizing radiation. Research is being conducted on the mechanisms underlying radiation-induced release of histamine from mast cells and the role of calcium in this process.

The Immunological Chemistry Division's research efforts are concerned with studies on the interaction of stromal tissue with hemopoietic cells and its importance in postirradiation hemopoietic regeneration. A series of studies is being conducted on the isolation of hemopoietic and progenitor cells and their potential as modifiers of radiation injury. The Fluorescence Activated Cell Sorter is used to obtain a pure population of hemopoietic and progenitor cells. A mutant mouse model extremely sensitive to radiation is also being used in these studies.

CYCLIC NUCLEOTIDE LEVELS IN CEREBROSPINAL FLUID OF THE PRIMATE: EFFECTS OF RADIATION

Principal Investigators: G. N. Catravas, S. J. Wright, Jr., P. J. Trocha, and J. K. Takenaga

Cyclic adenosine monophosphate (AMP) was originally thought to mediate positive and negative regulatory actions in the cell through bidirectional changes in its cellular levels. With the discovery of cyclic guanosine monophosphate (GMP), evidence indicated a number of biologic systems in which cyclic AMP and cyclic GMP seem to impose contrasting, often antagonistic regulatory influences (1). Increases in cerebrospinal fluid cyclic AMP levels have resulted from in vitro and in vivo administration of putative adrenergic neurotransmitters and other experimental clinical conditions (2). Daily fluctuations in cerebrospinal fluid cyclic AMP levels in the rhesus monkey have also been observed (3). Since little information is available on the radiation-induced changes in cyclic AMP and cyclic GMP in cerebrospinal fluid, we decided to investigate the effects of ionizing radiation on levels of these nucleotides.

Silastic Pudenz catheters were chronically implanted in the fourth ventricle of male cynomolgus monkeys (2.5-3.5 kg). The catheters were connected to compressible polyethylene Ommaya reservoirs placed subcutaneously over the occiput. This method permitted sterile aspiration of 0.5-1.0 ml cerebrospinal fluid in the awake animal. Before irradiation, duplicate baseline samples were taken 24 hours apart after repeated reservoir pumping to ensure good mixing with cerebrospinal fluid in the ventricle. This was verified by injecting 3 mCi of technetium-99m followed by repeated manual compression and passive filling of the reservoir. Radiocisternography of the ventricular system revealed cerebrospinal fluid exchange between the fourth ventricle, the posterior fossa, and the upper spinal subarachnoid space. The animals were then exposed, head only, to either 300 or 900 rads of 6.5 MeV bremsstrahlung from the AFRRI linear accelerator. The average dose rate was 70 rads per minute. Cerebrospinal fluid samples were taken at 1, 24, 48, and 72 hours after irradiation, using hypodermic syringe and needle. Sample collection time was at 1300 hours. Results are shown in Table 1.

Significant increases in levels of both cyclic AMP and cyclic GMP were observed after irradiation. These increases were more pronounced in the animals that received 900 rads bremsstrahlung than in the animals exposed to 300 rads, especially in the levels of cyclic GMP.

It is not clear from these experiments whether the observed changes in cerebrospinal fluid cyclic AMP and cyclic GMP reflect changes in brain cyclic nucleotides, or changes in their transport from brain to cerebrospinal fluid, or degradation of cyclic nucleotides within the intercellular spaces of the brain, or a combination of these factors. Additional experiments are necessary to elucidate these possibilities.

Table 1. Cyclic AMP and Cyclic GMP Levels (pmoles/ml Cerebrospinal Fluid)

	Preirradiation (Hours)	Postirradiation (Hours)			
	24	1	24	48	72
Monkey #1 (300 rads):					
Cyclic AMP	24.5	37.5	36.5	42.0	29.4
Cyclic GMP	3.9	5.9	4.7	4.7	6.4
Monkey #2 (900 rads):					
Cyclic AMP	30.5	60.0	55.1	55.3	85.0
Cyclic GMP	5.9	18.7	16.0	30.7	40.0
Monkey #3 (900 rads):					
Cyclic AMP	32.6	55.0	50.9	44.3	—
Cyclic GMP	6.9	33.3	14.7	8.0	—

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EFFECT OF LOW-LEVEL RADIATION ON THE PRENATAL MAMMAL AND THE JUVENILE MAMMAL

Principal Investigators: D. F. McClain, G. N. Catravas, and J. M. Mitchell

After low-level ionizing irradiation of the prenatal animal and the newborn animal, alteration occurs in the normal patterns of change of certain enzymes during development. Protein synthesis and nucleic acid metabolism are two basic systems affected, probably resulting from interference with normal translation and transcription after radiation-induced chromosomal damage. Ribonucleic acid (RNA) polymerase is of particular interest because of its pivotal role as a link between genetic information and production of metabolic enzymes. It is a highly controlled enzyme. Interference with any of the many initiation, elongation, and termination factors involved in RNA synthesis could profoundly affect RNA polymerase activity. RNA polymerase might thus serve as a sensitive indicator of chromosomal alterations in the cell.

Initial experiments were run in which a dose of 100 Roentgen of X radiation was administered to litters of 1-day-old rats. Intact nuclei were extracted from their brains and livers, and nuclear RNA polymerase activities were measured over a period of 21 days after birth.

Results of these experiments indicate a precocious increase in activity of RNA polymerase in the irradiated animals compared to nonirradiated controls. Maximum activity occurred within the first 7 days after irradiation in both liver and brain. Normal activities were not observed in the irradiated animals until at least 20 days after exposure.

This increased activity may reflect induced synthesis of a new enzyme, activation of inactive forms of the enzyme, or conversion of active enzyme to a form with greater specific activity. Experiments are continuing, to test each hypothesis.

MODULATION OF IMMUNOSUPPRESSION RESULTING FROM IRRADIATION

Principal Investigators: J. F. Weiss and K. F. McCarthy, *AFRRI*
Collaborators: M. A. Chirigos, W. A. Stylos, and R. M. Schultz, *National Institutes of Health*
Technical Assistance: W. W. Wolfe

Information is needed on the effects of radiation on various organs and cells involved in immune defense and their interrelationships. This information is important in understanding radiation injury related to nuclear weapons effects, including collateral damage and therapeutic doses of radiation. This work unit also investigated a new area of radioprotection: the use of immune adjuvants to protect against whole-body irradiation or partial-body irradiation.

Among the experiments initiated, one study showed that cytotoxic macrophages were induced after irradiation. Whole-body X irradiation (200-800 rads) and subcutaneous cyclophosphamide treatment (150-500 mg/kg) were studied for their influence on the ability of adjuvants to induce cytotoxic macrophages in vivo. Surprisingly, radiation alone or cyclophosphamide therapy alone produced growth-inhibiting macrophages whose function peaked within 2 days after treatment (Figure 1). When adjuvants such as Bacillus Calmette-Guérin (BCG), pyran copolymer, or glucan were administered intraperitoneally within 2 hours after sublethal X irradiation (600 rads), the adjuvant-induced cytotoxic function was depressed but not ablated. In addition, when non-induced peritoneal macrophages were obtained 6 days after lethal X irradiation (800 rads), their ability to be activated in vitro by lymphokine or fibroblast-derived interferon preparations was depressed only slightly at all tested concentrations of inducer. When BCG, pyran, or glucan was administered intraperitoneally concurrently with subcutaneous cyclophosphamide, only the ability of BCG to activate macrophages was markedly reduced. This indicates separate mechanisms for induction of tumoricidal macrophages. A better understanding of the interaction of the chemotherapeutic and/or radiation regimens with the adjuvants that affect macrophage function may be instrumental to rationalized immunotherapy protocols.

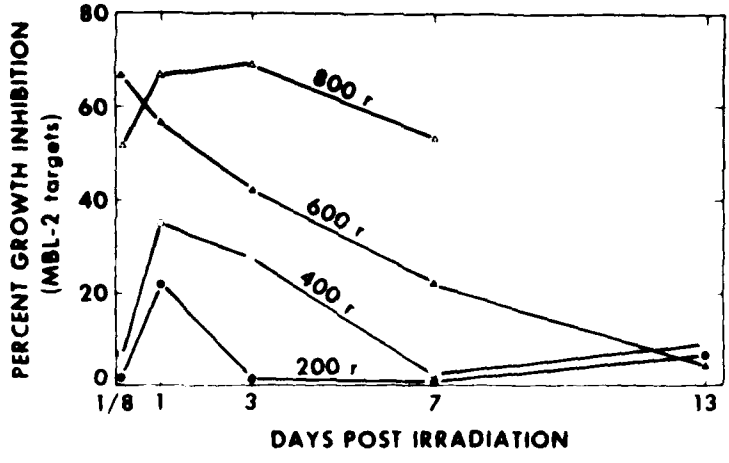

Figure 1. Chronologic appearance of cytotoxic macrophages in mice after whole-body X irradiation. (Reprinted with permission from R. M. Schultz et al., Cell Immunology 38: 302-309, 1978.)

PASSIVE TRANSFER OF CELL-MEDIATED IMMUNITY BY TRANSFER FACTOR

Principal Investigators: J. M. Mitchell, M. Porvaznik, and G. N. Catravas, *AFRRI*
P. Klesius, *USDA Regional Parasite Laboratory, Auburn, Alabama*

Transfer factor is a compound of small molecular weight derived from lymphocytes of animals that exhibit delayed hypersensitivity to a specific antigen. Transfer factor, when injected into a nonsensitized animal, results in a transfer of hypersensitivity (1). Transfer factor derived from lymphocytes of bovine sensitized to cell membranes of B16 mouse melanoma was found to significantly extend survival time of C57BL/6 and B6D2F1 female mice when 200 μg/g body weight of transfer factor was injected intraperitoneally 3 days before challenge with one million viable B16C3 mouse melanoma cells. Additional doses of transfer factor given after the challenge did not extend survival time, and appeared to shorten it when given 21 days post-challenge. Neither single nor multiple doses of transfer factor extended the survival time of male mice of either strain.

Treatment of female mice with transfer factor 1 day after receiving 300 rads of cobalt-60 and subsequent challenge with melanoma cells did not extend survival time compared to either irradiated mice without transfer factor or sham-irradiated mice without transfer factor. Analysis was made of (a) the survival curves of mice that responded to transfer factor treatment and (b) the in vitro stimulation of lymphocytes from transfer factor-treated mice. It was found that only 20% of the mice responded to transfer factor. These data indicate that some animals receive beneficial stimulation of the cell-mediated immune system under normal conditions but that this beneficial response is lost after irradiation.

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STEROL METABOLISM IN NEURAL TISSUE

Principal Investigators: J. F. Weiss and C. R. Dobbs, *AFRRI*
Collaborators: H. Cravioto and F. Drnovsky, *New York University Medical Center*
Technical Assistance: W. W. Wolfe

The high concentration of cholesterol in nervous tissue and its localization in cell membranes--notably myelin--indicate an important role for this sterol and related compounds in the growth, maturation, and metabolism of the brain. However, very little has been known not only about sterol function but also about sterol composition and pathways of biosynthesis and metabolism in the brain. Introduction of drugs that inhibit specific steps of cholesterol biosynthesis in the nervous system has made possible a different approach to solving these complex problems. This involves inducing selective accumulation of precursors that can be separated and identified by

techniques such as gas chromatography-mass spectrometry. Although sterol metabolism is slower in mature brain compared to developing and neoplastic brain, sterol synthesis and turnover do occur in mature nervous tissue and can be affected by disease, injury, or drugs. For example, ionizing radiation appears to block brain cholesterol synthesis, and this effect can be modified by radioprotective agents or radiosensitizers.

AY-9944 is a drug that alters cholesterol metabolism, and a major effect of treatment with AY-9944 is the accumulation of 7-dehydrocholesterol in tissues with active cholesterol metabolism, including the developing brain and brain tumors. The effect of AY-9944 on sterol composition, tumor growth, and histology was studied using various model systems for rat brain tumor (ethyl nitrosourea-induced primary tumors, subcutaneously grown tumors, and tumors in tissue culture derived from primary gliomas). Growth inhibition and accumulation of cholesterol precursors occurred in varying degrees in all tumor systems. In cultured glial cells, maximum accumulation of 7-dehydrocholesterol and drug toxicity occurred when cells were grown in a medium devoid of cholesterol. Rats that were transplacentally exposed to ethyl nitrosourea were treated with AY-9944 (intravenously, twice a week, 5 mg/kg body weight) beginning at 7 months of age until tumor appearance or death. Compared to non-treated littermates, rats treated with AY-9944 had increased survival time ($p = 0.05$) (Figure 1). Median latency period for tumor development was 302 days for the non-treated group and 437 days for the treated group. These experiments support a different concept of brain tumor treatment consisting of either blockage of the increased rate of cholesterol synthesis found in brain tumors or replacement of cell membrane cholesterol by other sterols. However, the possibility that tumor growth may be inhibited by drug effects unrelated to sterol metabolism cannot be excluded at the present time.

Further studies will determine whether changes in neural tissue sterols after drug treatment result in modification of tissue radiosensitivity.

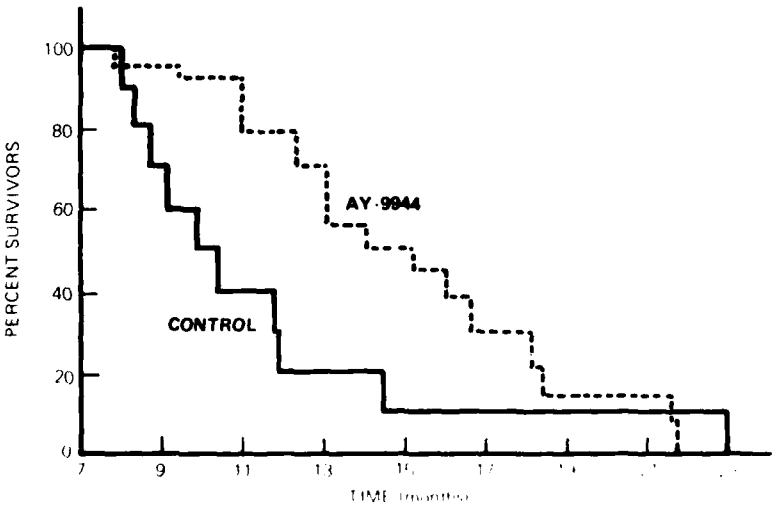

Figure 1. Effect of AY-9944 on rat survival after transplacental ethyl nitrosourea treatment (From J. F. Weiss et al., Journal of Neuropathology and Experimental Neurology 37: 706, 1978)

RADIATION AND SERUM METALS

Principal Investigators: W. P. Bradley and J. F. Weiss, *AFRRI*
Collaborator: P. O. Alderson, *Johns Hopkins Hospital*
Technical Assistance: C. J. Morrisey and C. L. Harding

Serum metal concentrations have been observed to change after irradiation by increase in levels of copper, zinc, and iron. Small doses of radiation also are reported to affect whole-body retention and serum binding of the radionuclide gallium-67. Investigation into radiation effects on serum metal concentrations was begun at AFRRI with early emphasis on gallium-67. Results indicated that the decreased gallium-67 retention and serum binding seen after whole-body irradiation are related, at least in part, to the saturation of transferrin by increased levels of circulating iron (1).

Further studies showed that other alterations of iron metabolism, such as iron deficiency, also affect biodistribution and tumor uptake of gallium-67 in rats. To investigate this, 20 weanling Sprague Dawley rats were maintained for 6-8 weeks on a low-iron diet. Eighteen littermates were maintained on a normal iron diet to serve as controls. Animals received 10 μCi of gallium-67 citrate, and urine and feces were collected for 48 hours. The animals were then sacrificed, tissue samples were obtained, and serum iron and unsaturated iron-binding capacity (UIBC) were measured. Accumulation of gallium-67 in the liver and spleen (percent injected dose per gram) was markedly increased in iron-deficient animals, and urinary excretion was reduced. Tumor uptake was not significantly different in iron-deficient and control animals, but tumor-to-blood ratios were elevated ($p < 0.001$) in the iron-deficient animals because of low blood levels of gallium-67. Liver and spleen accumulation of gallium-67 correlated significantly ($p < 0.001$) with unsaturated iron-binding capacity (Figure 1). Results show that iron deficiency alters the distribution of gallium-67 citrate and suggest that the variable liver-spleen uptake seen in clinical gallium-67 images may be explained, in part, by changes in serum iron and unsaturated iron-binding capacity.

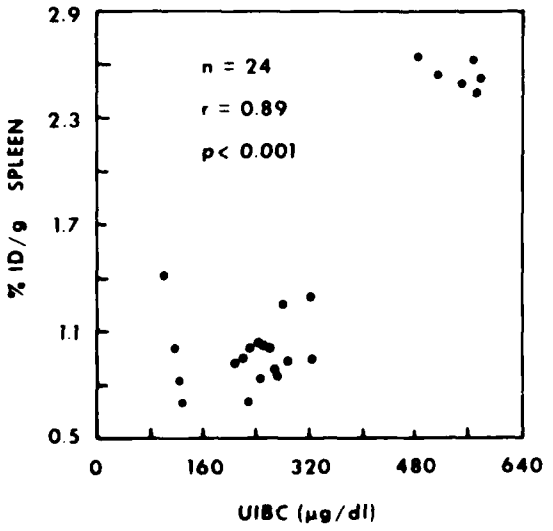

Figure 1. Correlation of unsaturated iron-binding capacity (UIBC) with uptake of gallium-67 (% ID/g) in spleens of normal rats and iron-deficient rats (From W. P. Bradley et al., Journal of Nuclear Medicine 20: 243-247, 1979)

It is evident that serum binding and biodistribution of gallium-67 are closely related to iron status. Measurements of either gallium-67 serum binding or serum iron may provide information about radiation exposure, but it is not clear which analysis is a better biological indicator of radiation damage. Future studies will aim at determining the effect of irradiation on other serum and urine metal ion concentrations, with emphasis on the mechanism of observed alterations and their potential use as biological dosimeters.

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PROPERTIES OF SERUM GLYCOPROTEINS AFFECTED BY RADIATION

Principal Investigators: J. F. Weiss, W. P. Bradley, and C. E. Elhardt, *AFRRI*
Collaborators: P. B. Chretien and A. M. Baskies, *National Institutes of Health*
Technical Assistance: L. R. Wooldridge, K. M. Hartley, and W. W. Wolfe

Elevations in serum glycoproteins and protein-bound carbohydrates are significant consequences of radiation damage, trauma, and certain diseases such as cancer. Thus it is important to determine the diagnostic, prognostic, and functional significance of the glycoprotein elevations in various injuries and diseases. Background information on levels of serum glycoproteins and protein-bound carbohydrates in cancer patients and normal persons have been published (1,2).

Further studies (3,4) indicated that serum glycoproteins may be biochemical markers of immune status and can be useful in assessing immunosuppressed persons. The following discusses the changes in serum glycoproteins in patients during radiotherapy.

There is increasing evidence that normal serum proteins, such as the acute-phase proteins, affect immune responses in vitro. Acute-phase proteins are elevated in experimental animals after radiation exposure. The acute-phase proteins α_1-acid glycoprotein, α_1-antitrypsin, haptoglobin, and C-reactive protein increase in patients during tumor growth whereas other proteins, α_2HS-glycoprotein and prealbumin, are depressed in cancer patients. These proteins were determined in patients being treated for head and neck cancer and were compared to immune parameters (lymphocyte reactivity to phytohemagglutinin and T-cell levels).

Increases in acute-phase proteins and depressions in α_2HS-glycoprotein and prealbumin were greater in patients with regionally metastatic tumors than in patients with localized tumors. In a group of patients with localized tumors before or after current radiotherapy (n = 41), serum proteins and immune parameters were plotted

versus radiation dose. Levels of α_1-acid glycoprotein correlated significantly with radiation dose ($p < 0.05$). There were parallel depressions of α_2HS-glycoprotein and T-cell levels as radiation dose increased. Of the serum proteins studied, α_2HS-glycoprotein best reflected the immune parameters. The α_2HS-glycoprotein correlated with T-cell levels in nontreated patients ($n = 19$, $p < 0.05$) and in patients during radiotherapy ($n = 43$, $p < 0.01$) but not with T-cell levels in a group of healthy persons ($n = 48$) or in patients with no evidence of disease ($n = 17$). These results suggest that specific serum alpha globulins may provide insight into the effect of radiotherapy, including its immunosuppressive action.

Another investigation provided additional evidence that α_2HS-glycoprotein was the best biochemical correlate of both in vivo and in vitro assays of cell-mediated immunity. For example, levels of serum α_2HS-glycoprotein correlated positively with delayed cutaneous hypersensitivity to dinitrochlorobenzene (Figure 1).

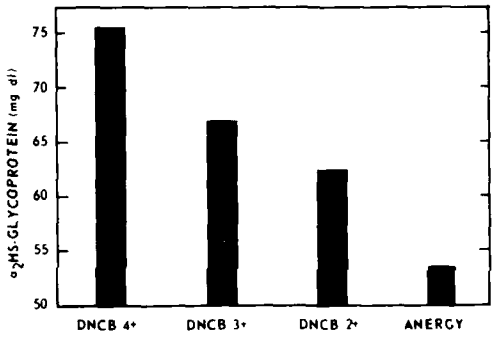

Figure 1. Delayed cutaneous hypersensitivity to dinitrochlorobenzene (DNCB). Data from ref. 4.

Progress was also made in improving assays for assessment of disease. Factors affecting serum sialic acid (proposed as a biochemical indicator of radiation damage) were studied in detail, as described in the following.

Serum acute-phase proteins show correlations with both tumor burden and immune competence in cancer patients. We investigated serum sialic acid as a measure of the acute-phase response as well as various factors that may affect sialic acid levels. Total serum sialic acid determined by an improved semi-automated method utilizing the thiobarbituric acid reaction was correlated with serum protein levels determined by radial immunodiffusion. In 50 healthy persons, serum sialic acid correlated with haptoglobin ($r = +0.71$, $p < 0.001$) and α_1-acid glycoprotein ($r = +0.39$, $p < 0.01$). In patients with solid malignancies studied either before treatment ($n = 100$) or during radiotherapy ($n = 115$), sialic acid correlated with haptoglobin, α_1-acid glycoprotein, and α_1-antitrypsin ($r = +0.49$ to $+0.90$, $p < 0.001$). We calculated that these three acute-phase proteins account for more than half of the increase in serum globulin and that they are the principal source of increased sialic acid observed in cancer patients. In 120 nontreated cancer patients whose serum was positive for C-reactive protein, the sialic acid and C-reactive protein levels correlated ($r = +0.63$, $p < 0.001$). This further indicated the significant relation of serum sialic acid to the acute-phase response. Evidence of a relation to immune parameters was shown by a negative

correlation with T-cell levels ($r = -0.27$, $p < 0.05$) in nontreated patients with solid tumors. Studies on 400 persons showed that sialic acid levels are affected by age (in healthy persons), smoking history, tumor burden, tumor histology, and clinical tumor recurrence (Figure 2). Precise determination of serum sialic acid by automated analysis provides information that correlates with clinical status of cancer patients and thus may be useful as a monitor of clinical course and response to treatment.

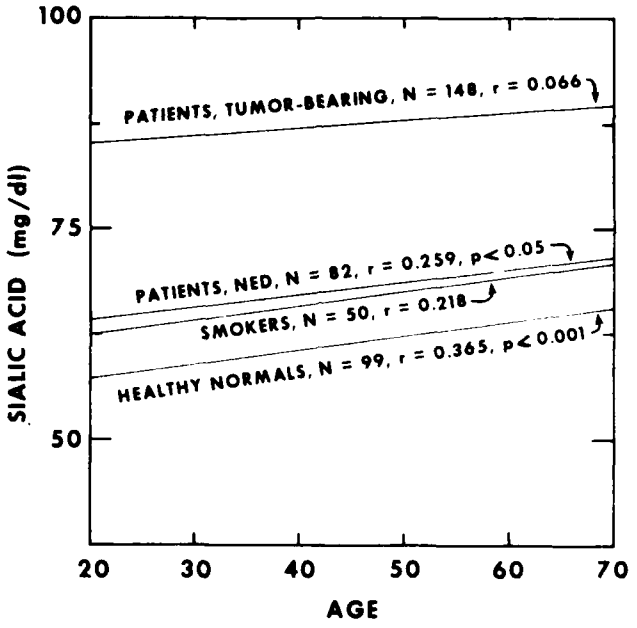

Figure 2. Effect of age on sialic acid levels (From J. F. Weiss et al., Proceedings of American Society of Clinical Oncology 19: 390, 1978 and from unpublished data)

Current emphasis in our studies of serum glycoproteins is placed on elucidating the hypothesized immunosuppressive (or immunostimulatory) effects of certain serum proteins, with the practical aim of diagnosing and treating immunosuppression such as that accompanying radiation exposure.

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PROSTAGLANDINS, LYSOSOMES, AND RADIATION INJURY

Principal Investigators: P. J. Trocha and G. N. Catravas

Prostaglandins and lysosomal enzymes have been shown to be altered after tissue injury (1), yet no studies have simultaneously monitored lysosomal enzyme activities and prostaglandin levels in animals exposed to ionizing radiation. Therefore the present investigation was designed to determine the relationship between lysosomal enzymes and prostaglandin levels in irradiated tissues.

Sprague-Dawley rats (weighing 75-125 g) were exposed bilaterally to 1000 rads of gamma radiation at a dose of 500 rads/min. At designated intervals after irradiation, the rats were sacrificed. Spleen and liver tissues were rapidly excised and frozen with liquid nitrogen except for portions (0.05 to 0.1 g) of each fresh tissue sample. The fresh spleen and liver aliquots were gently homogenized. A portion of each homogenate was centrifuged at 12,000 x g in order to obtain a supernatant free of lysosomes. The uncentrifuged tissue homogenates and the 12,000-x-g supernatants were assayed for β-glucuronidase activity.

Isolation and assay of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the frozen tissues were performed by standard techniques (2).

A transient two- to fourfold increase in $PGF_{2\alpha}$ levels occurred in spleen and liver tissues 5 to 12 hours after irradiation of the animals. Also during this time there was a twofold rise in total cellular β-glucuronidase activity in the spleen and a transient increase in percentage of β-glucuronidase activity in the 12,000-x-g supernatant from 20% to 45%. However, there was no observed increase in enzyme activity or leakage from the lysosomes in liver tissue. $PGF_{2\alpha}$ levels in the spleen and liver again rose markedly in the exposed animals to maximum values of 1800 pg/g spleen tissue and 500 pg/g liver tissue at 4 and 7 days, respectively, before returning to normal values of 175-250 pg/g tissue at 11 days. The lysosomal β-glucuronidase activities and

leakages were further noted to increase 300% in the spleen and 25% in the liver during these later prostaglandin elevations.

Changes in prostaglandin $F_{2\alpha}$ levels and β-glucuronidase activities occurred at nearly the same times and with similar magnitudes. These changes indicate that either the increase in lysosomal enzyme activities and leakage of lysosomes cause $PGF_{2\alpha}$ to rise or an elevation in prostaglandin concentration results in the activation and release of enzymes from the lysosomes.

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EXPERIMENTAL HEMATOLOGY DEPARTMENT

PROGRAM: Postirradiation Protection From Fatal Infections

Exposure to ionizing radiation doses of 100-200 rads damages or destroys bone marrow cells, resulting in reduction of or cessation in production of granulocytes, macrophages, and platelets, which are the first and major defense against infectious bacteria and their toxins. With increasing radiation doses, these infections result in fatalities. Infections and fatalities can be decreased by procedures that protect bone marrow cells from these effects, or enhance their endogenous production postirradiation, or temporarily supply functional granulocytes until the radiation-damaged bone marrow recovers. In addition, successful treatment is promoted by means that would prevent the invasion of intestinal gram-negative bacteria into other tissues and organs of irradiated persons or at least reduce the concentration of those bacteria. Successful treatment would permit exposure of persons to higher radiation doses if demanded by extreme military situations and also the use of enhanced nuclear weapons since it would raise the radiation dose that would cause 5% fatalities.

The Departmental program is divided into five project groups, each researching a specific area.

PROJECT GROUP 1: Enhancement of White Cell Production Postirradiation

This project group consists of a number of projects to elucidate the interaction of (a) humoral substances released by functional white blood cells in loci of inflammation and infection and (b) the primitive precursor cells for increased production of adult functional cells. Of particular interest is the task of learning how to manipulate the radiation-injured precursor system by molecular engineering in order to enhance white cell production to fight against invading bacteria and their toxins. Significant progress has been made in determination of humoral and cellular interactions as well as in delineation of various cell types of the precursor system.

PROJECT GROUP 2: Studies of Origin and Prevention of Infection Postirradiation

These projects deal with experimental designs to discover possible routes of bacterial invasion postirradiation, means of preventing this occurrence, and means of increasing defense against the bacteria and their toxins in a radiation-injured organism. Progress was made in all these projects.

PROJECT GROUP 3: Combined Injury

Military analysts have estimated that, in a future atomic war, more than 70% of the casualties will suffer certain injuries in addition to those caused by ionizing radiation. The greater percentage of those injuries will be wounds or burns. German and Russian studies using mice or dogs indicate that presence of open wounds after radiation will increase the number of fatalities whereas immediate suturing of open wounds will not. Unfortunately, because of septic conditions, surgeons usually postpone suturing of wounds. Since the hematopoietic system is involved in wound healing, it is important to study that system's functional status in the irradiated animal.

Significant findings were made on the effect of ionizing radiation on immunocompetent cells in wounded animals. Essentially, B and T cell responses to specific mitogens were reduced. In addition, it was observed that lysosomal enzymes released from phagocytic cells may contribute significantly to stress and tissue injury in irradiated persons.

PROJECT GROUP 4: Physiological Assessment of Fresh and Cryopreserved Granulocytes and Macrophages Used for Postirradiation Transfusion

Bone marrow exposed to radiation doses of 350-500 rads still has capability of recovering if the animal or human does not die from infection. The best treatment is infusion of compatible granulocytes. These projects will establish (a) simple and rapid test procedures for *in vitro* evaluation of granulocytes used in postirradiation therapeutic transfusions and (b) a correlation between new test procedures and the old, more cumbersome, more time-consuming tests. It was observed that the new, rapid tests of cell integrity were as accurate as the previously used time-consuming procedures.

Design and successful testing of the enlarged rotor system for granulocyte separation were completed. Experimentation indicated that clinically usable numbers of granulocytes can be isolated by counterflow centrifugation-elutriation. Initial experiments of freshly isolated granulocytes versus leukapheresis granulocytes indicate that the cells are as effective as, if not more effective than, cells obtained by leukapheresis alone.

PROJECT GROUP 5: Transplantation of Bone Marrow Cells Into Lethally Irradiated Animals

Once radiation destroys the bone marrow completely, no endogenous recovery is possible. In such a case, bone marrow transplantation by genetically identical persons is the only means of treatment and recovery. However, genetically identical cells are usually not available (with exception of those from identical twins), and transplantation of incompatible bone marrow results in death. The objective is to modify incompatible bone marrow cells by removal of specific T-lymphocytes. Early studies showed that removal of these T-lymphocytes results in significant increase of postirradiation survival in mice.

Specific tests were designed to identify lymphocytes, called suppressor cells, that appear to be capable of inactivating other lymphocytes, called killer cells. Killer cells attack tissues and organs consisting of cells that do not have specific cellular antigenic markers. These are the cells that initiate organ transplant rejection or graft-versus-host reaction in bone marrow transplants postirradiation.

ENDOTOXIN-INDUCED ALTERATIONS IN CANINE GRANULOPOIESIS: COLONY-STIMULATING FACTOR, COLONY-FORMING CELLS IN CULTURE, AND GROWTH OF CELLS IN DIFFUSION CHAMBERS

Principal Investigators: T. J. MacVittie and R. I. Walker
Technical Assistance: R. T. Brandenburg, J. L. Atkinson, and E. G. McCarthy

Salmonella typhosa endotoxin injected into dogs produced elevated levels of plasma colony-stimulating factor, transient leukopenia followed by leukocytosis, and stimulation of marrow granulopoiesis and mobilization of granulocyte-macrophage progenitor cells into the peripheral circulation. The number of marrow granulocyte-macrophage progenitor cells (CFU-c) decreased to 65% of the control number within 6 hours, returned to control levels by 24 hours, and increased to 370% of the control number by 48 hours after endotoxin (Figure 1). The granulopoietic response was supported by (a) a concomitant increase in the myeloid-erythroid ratio, (b) an increased fraction of marrow-derived CFU-c susceptible to tritiated-thymidine suicide, and (c) increased granulo-monocytopoietic activity of marrow-derived and peripheral blood-derived cells grown in diffusion chamber cultures (Figure 2).

These results are consistent with the concept that endotoxin-induced colony-stimulating factor is a physiologic regulator of canine granulopoiesis. The canine marrow responded to endotoxin with a significant increase of concentration of marrow-derived granulocytic progenitors and with mobilization of granulocyte-macrophage progenitors into the peripheral circulation.

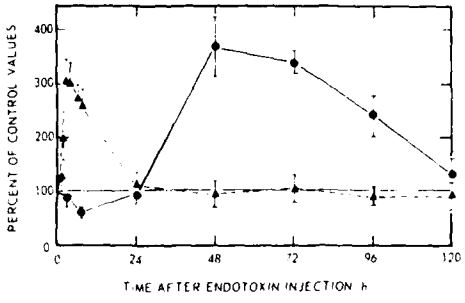


Figure 1. Alterations in canine marrow CFU-c concentration (●) and plasma colony-stimulating factor levels (▲) as percent of control values following injection of *Salmonella typhosa* endotoxin. Values (± SEM) are means of seven replicate experiments.

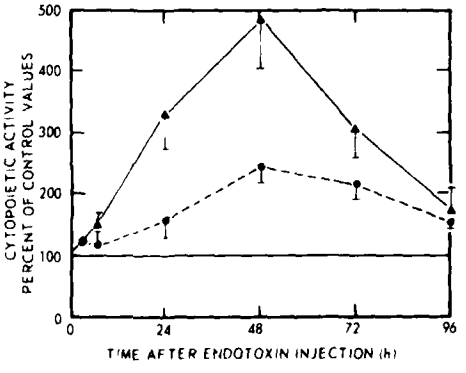


Figure 2. Alterations in cytopoietic activity (number of total nucleated cells harvested at day 7 of culture divided by number of total nucleated cells inoculated per chamber) of diffusion chamber progenitor cells derived from canine marrow (●) and peripheral blood leukocytes (▲). Values (± SEM) are means of seven replicate experiments.

CANINE GRANULOPOIESIS: ALTERATIONS INDUCED BY SUPPRESSION OF GRAM-NEGATIVE FLORA

Principal Investigators: T. J. MacVittie and R. I. Walker
Technical Assistance: J. L. Atkinson, R. T. Brandenburg, and E. G. McCarthy

We investigated alterations in canine granulopoiesis following suppression of bacterial flora by antibiotic treatment. Beagles were decontaminated by an antifungal and antibiotic regimen (Figure 1) in sterile isolation with laminar air flow. Skin cultures and fecal specimen cultures were usually negative after 4 days.

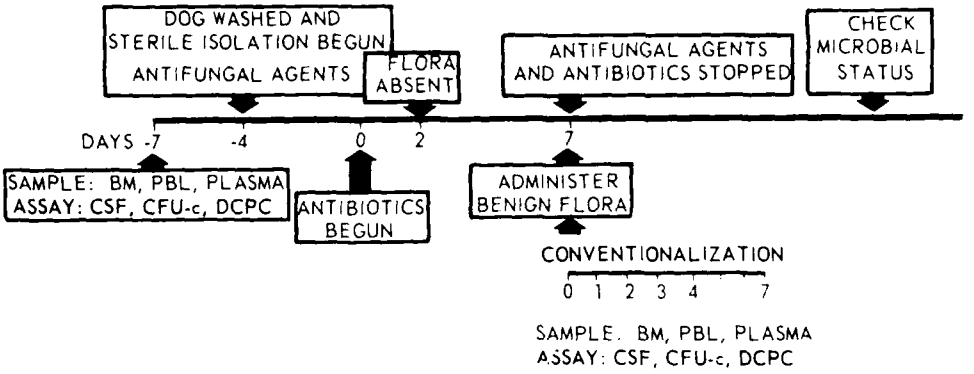


Figure 1. Experimental protocol for suppression of intestinal flora, subsequent conventionalization, and determination of granulopoietic status. BM, bone marrow; PBL, peripheral blood leukocytes; CSF, colony-stimulating factor; CFU-c, granulocyte-macrophage progenitor cells; DCPC, diffusion chamber progenitor cell

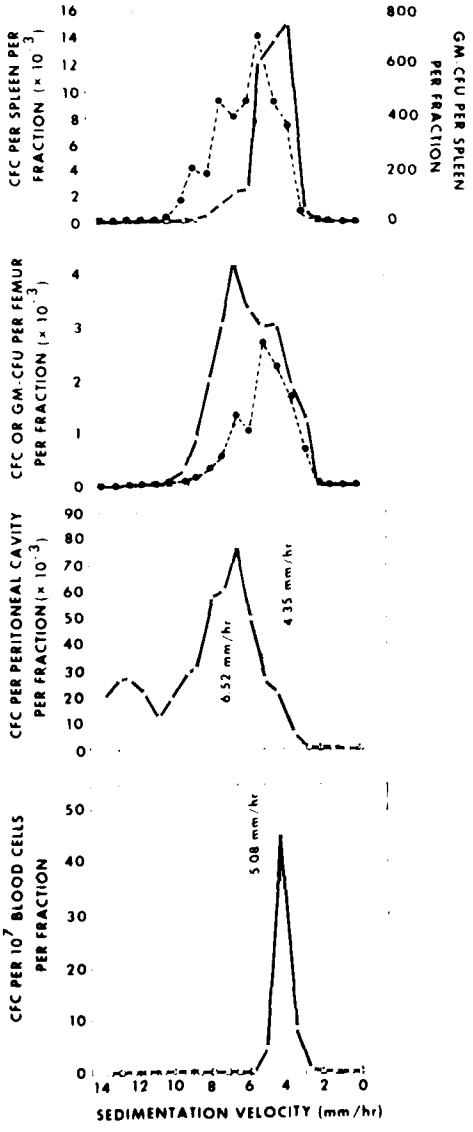
Reduction of gram-negative bacteria resulted in significant decrease of levels of plasma colony-stimulating factor, marrow granulocyte-macrophage progenitor cell (CFU-c) concentration, and cytopoietic activity of marrow-derived diffusion chamber progenitor cells. The period of conventionalization was characterized by a significant although delayed increase in plasma colony-stimulating factor as well as by marked increases in marrow CFU-c concentration and cytopoietic activity of marrow diffusion chamber progenitors. There was also mobilization of marrow diffusion chamber progenitors into the circulation. All parameters returned to control values within 7 days of conventionalization.

These data support the hypothesis that the gram-negative bacteria of the gut play a significant role in regulation of normal canine granulopoiesis.

VELOCITY SEDIMENTATION STUDIES ON MONOCYTE-MACROPHAGE COLONY-FORMING CELLS FROM MARROW, SPLEEN, BLOOD, AND PERITONEAL EXUDATE

Principal Investigator: K. F. McCarthy
Collaborator: T. J. MacVittie
Technical Assistance: E. G. McCarthy and P. W. Jones III

We report and compare (a) velocity sedimentation profiles (Figure 1) of colony-forming cells that generate mononuclear phagocytic progeny in vitro (1-3) to (b) velocity sedimentation profiles of granulocyte-macrophage progenitor cells.



It was found that granulocyte-macrophage progenitor cells, whether from marrow or spleen, are characterized by similar velocity sedimentation profiles with a peak sedimentation value of about 5.2 mm/hour. In contrast is the heterogeneity of the colony-forming cell population, composed of two subpopulations with peak sedimentation values of about 4.1 mm/hour and 6.5 mm/hour (4). The 6.5-mm/hour colony-forming cell subpopulation is found in marrow and possibly in peritoneal exudate, and the 4.1-mm/hour colony-forming cell population is found in marrow, spleen, and peripheral blood.

Figure 1. Representative sedimentation velocity profiles of granulocyte-macrophage progenitor cells (GM-CFC) ●---● and colony-forming cells (CFC) —— from various tissues when cells in various fractions were assayed in presence of 3.0% pregnant mouse uterine extract

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POPULATION SIZES OF GRANULOCYTE-MACROPHAGE AND MONOCYTE-MACROPHAGE COLONY-FORMING CELLS IN *Sl/Sl^d* MICE

Principal Investigator: K. F. McCarthy
Collaborator: T. J. MacVittie

Sizes of granulocyte-macrophage (CFU-c) and monocyte-macrophage (CFC) progenitor cell populations were measured and compared in Sl/Sl^d mice and +/+ mice.

In the marrow, the CFU-c and CFC population sizes were 40% and 67%, respectively, of the +/+ marrow CFU-c and CFC population sizes. There was no difference in sizes of these two cell populations in the spleens or thymi of Sl/Sl^d mice as compared to +/+ mice (Table 1). Furthermore, the marrow CFC population was found to be heterogeneous by velocity sedimentation. One of the marrow CFC subpopulations is characterized by a velocity sedimentation value of 5.05 mm/hour, and the other has a value of 6.30 mm/hour.

Table 1. CFU-c and CFC Per 10^6 Nucleated Cells and Per Organ From Marrow, Spleen and Thymus of *Sl/Sl*^{d} and +/+ Mice

Organ	Cellularity ($\times 10^{-7}$)	CFU-c/10^6 cells	CFU-c/Organ	CFC/10^6 cells	CFC/Organ
+/+					
Femur	2.4 ± 0.2*	640 ± 170	14,395 ± 3,522	2,890 ± 430	68,906 ± 11,792
Spleen	10.5 ± 0.8	4.5 ± 1.8	507 ± 188	275 ± 163	31,179 ± 11,444
Thymus	7.9 ± 1.3	—	—	10 ± 6	557 ± 286
Blood†	1.0 ± 0.2	—	—	—	—
Sl/Sl^d					
Femur	1.1 ± 0.2	530 ± 80	5,692 ± 1,065	3,970 ± 600	45,953 ± 8,780
Spleen	13.3 ± 1.1	4.8 ± 1.8	584 ± 208	324 ± 163	44,837 ± 19,629
Thymus	3.1 ± 1.1	—	—	32 ± 13	543 ± 202
Blood	1.0 ± 0.2	—	—	—	—

* Mean ± S.E.
† per ml

TIGHT JUNCTION DISRUPTION AND RECOVERY AFTER SUBLETHAL GAMMA IRRADIATION

Principal Investigator: M. Porvaznik

Ilea from sublethally irradiated, adult rats were prepared for freeze fracture and lanthanum tracer study to investigate alterations that occur in structure and function of the intestinal permeability barrier.

Using the freeze-fracture technique, it was determined that some of the tight junction structures are focally disrupted between days 1 and 5 postirradiation. Alterations in mean depth of the apical tight junction appeared to correlate with permeability of the epithelium to lanthanum tracer. Occurrence of tight junctional fragments over extensive areas between lateral membrane fracture faces was observed during the disruption and recovery phases. The fragments were manifested as linear and macular tight junctions extending basally from the apical tight junction (zonula occludens) as far as the basal lamina. These "proliferative" tight junction fragments were thought to be eventually removed by phagocytosis since numerous tight junctional fragments could be observed in cytoplasmic vesicles between days 3 and 7 after irradiation. Lanthanum tracer, added only to the intestinal lumen during fixation for electron microscopy, was found in extracellular spaces between some goblet cells and adjacent absorptive epithelial cells in preparations from irradiated rats. "Leaky" tight junctions were observed between days 1 and 7 postirradiation, which then returned to control levels.

BIOLOGIC PROPERTIES OF BACTERIAL LIPOPOLYSACCHARIDES TREATED WITH CHROMIUM CHLORIDE

Principal Investigator: S. L. Snyder
Collaborators: R. I. Walker, T. J. MacVittie, and J. M. Sheil

Exposure to large doses of radiation severely compromises a person's resistance to infection. Therefore, agents that nonspecifically enhance host resistance to bacterial or viral infections might increase survival after radiation exposure. Endotoxin (bacterial lipopolysaccharide, LPS) is an agent known to enhance host resistance to bacterial, viral, or fungal infection as well as to radiation injury. Unfortunately, the highly toxic properties of LPS severely limit its application as an agent for stimulating host defense mechanisms.

In this study we attempted to attenuate the toxic properties of LPS while leaving intact its potentially useful properties. Specifically we have shown that addition of small amounts of chromium chloride to a saline suspension of Salmonella typhosa LPS causes marked reduction in several biologic activities of this substance. These include toxicity, B-cell mitogenicity, plasma colony-stimulating activity, radioprotective effect, and induction of the dermal Shwartzman reaction. Nevertheless, LPS treated with chromium chloride was found to be at least as effective as nontreated LPS in enhancing the resistance of B6CBF1 mice to the lethal effects of Klebsiella pneumoniae infection (see Table 1) (1).

Table 1. Survival (7-Day) of B6CBF1 Mice Pretreated With LPS(W)[a] or Cr(III)-LPS(W)[b] Before Challenge With *Klebsiella Pneumoniae*[c]

Dose, μg/[d]	% survival after infection						Av % survival ± SEM	
	Expt. 1[e]		Expt. 2		Expt. 3			
LPS or Cr(III)-LPS	LPS	Cr(III)-LPS	LPS	Cr(III)-LPS	LPS	Cr(III)-LPS	LPS	Cr(III)-LPS
0[f]	20	20	0	0	10	10	10 ± 6	10 ± 6
0.01	30	40	20	40	90	60	47 ± 22	47 ± 7
0.10	60	70	90	90	90	90	80 ± 10	83 ± 7
1.0	40	80	30	60	90	90	53 ± 19	77 ± 9
10	70	90	50	90	60	80	60 ± 6	87 ± 3
100	20	40	20	30	0	60	13 ± 7	43 ± 9

[a] *Salmonella typhosa* lipopolysaccharide (Westphal preparation)
[b] Chromium-treated LPS(W)
[c] An amount of 10^6 bacterial/mouse
[d] A single injection of LPS (0.2 ml) of dose shown was given 24 h before subcutaneous challenge with *K. pneumoniae* (0.2 ml)
[e] Ten mice were used at each dose studied in experiments 1, 2, and 3
[f] Controls received 0.2 ml 0.9% saline

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ANTIBIOTIC DECONTAMINATION OF THE DOG AND ITS CONSEQUENCES

Principal Investigators: R. J. Walker, J. J. MacVittie, B. J. Sinha, P. F. Ewald, J. F. Egan, and G. J. McGuire

A regimen of isolation and decontamination was developed that effectively reduces the number of resident flora of the dog.

Bacterial counts in four dogs before treatment were 3.8×10^9 per gram of feces. No organisms were detectable in these same dogs after treatment, but the intestinal flora returned to slightly above normal levels by 1 week after treatment.

Decontamination was accomplished in a laminar air-flow system designed to minimize the area under controlled conditions (Figure 1). We determined the antibiotic sensitivities of 67 isolated organisms representing eight species or groups of bacteria recovered from the four dogs (Table 1). Then we developed a standardized antibiotic regimen consisting of bacitracin and neomycin administered as a dry powder in the food. The decontamination treatment apparently did not affect host metabolism because no alterations were found in serum levels of urea nitrogen, glucose, phosphate, total protein, chloride, sodium, potassium, serum glutamic oxalacetic transaminase, or serum glutamic pyruvic transaminase in the antibiotic-treated dogs. However, the decontamination process did reduce normal granulopoietic stimulation.

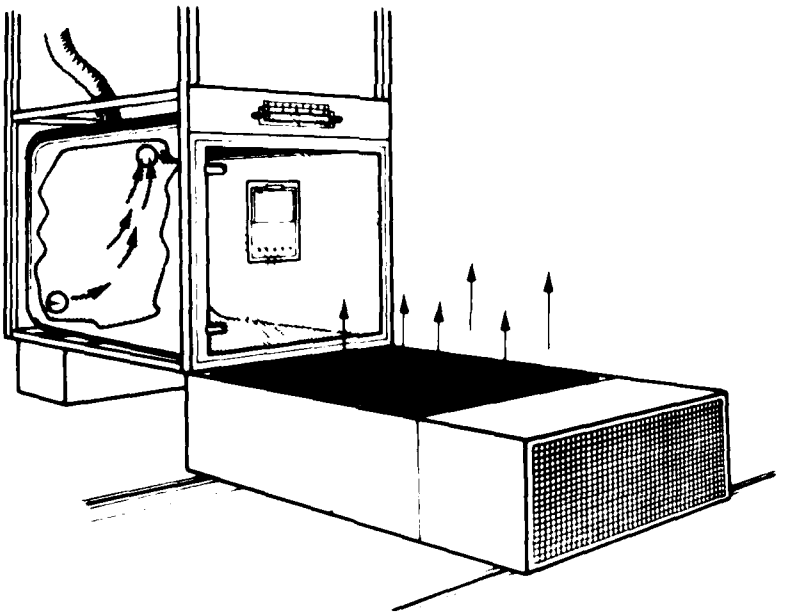


Figure 1. Isolation cage for dog. Sterile air is circulated within cage (curved arrows) by laminar air-flow unit mounted on cage top. Movable laminar air-flow unit provides sterile air in front of cage (vertical arrows).

Table 1. Bacteria Isolated From Four Dogs Before Decontamination and the Antibiotic Sensitivity of the Isolates

Bacterium	Number of isolates	Number of isolates antibiotic-sensitive[a]											
		S[b]	P	G	V	A	E	N	B	K	C	T	KA
Escherichia coli	28	15	27	0	28	0	0	0	26	1	0	0	0
Enterobacter cloacae	3	3	3	0	3	0	0	0	3	1	0	1	0
Enterococcus	13	9	5	7	1	0	1	13	0	0	9	12	11
Clostridium perfringens	8	8	0	8	0	0	0	8	0	0	4	0	6
Staphylococcus aureus	5	0	0	0	0	0	0	0	0	0	0	0	0
Klebsiella pneumoniae	4	4	4	0	4	0	0	0	4	0	0	0	0
Proteus mirabilis	3	0	1	0	3	0	2	0	3	0	1	3	0
Anaerobic diphtheroids	3	3	0	0	3	0	0	3	0	0	3	0	3
	67												

[a] Sensitivity determined with antibiotic-impregnated paper disks.

[b] S - streptomycin, P - penicillin G - gentamycin, V - vanocomycin; A - ampicillin, E - erythromycin, N - neomycin, B - bacitracin, K - keflin, C - colymycin, T - tetracycline, KA - kantrex.

ASSOCIATION OF LEUKOPENIA AND INTESTINAL PERMEABILITY WITH RADIATION-INDUCED SENSITIVITY TO ENDOTOXIN

Principal Investigators: R. I. Walker and M. Porvaznik

Sensitivity to the lethal effects of endotoxin is increased after exposure to ionizing radiation in the hematopoietic death range. We hypothesized that leukopenia and altered intestinal permeability may be causative factors of increased sensitivity to endotoxin after irradiation. The presence of leukocytes and platelets was correlated (Table 1) with sensitivity of male B6CBF1 mice to 0.25 mg of Salmonella typhosa

Table 1. Correlation of Leukopenia and Thrombocytopenia With Susceptibility to Endotoxin After 1000 Rads γ-Radiation

Time After Irradiation (days)	Mortality		Cell Counts		
	Dead/Total	Percent	WBC*	Platelets	#Mice
0	0/15	0	10,880	1,555,000	5
2	3/20	15	1,020	1,312,000	5
3	24/30	80	300	1,212,500	10
5	3/12	25	33	670,000	3
6	1/15	6.7	130	447,900	10
7	6/11	54	50	195,000	8
9	9/9	100	200	14,000	5
10	11/11	100	0	18,000	3

*White blood cells

endotoxin administered intraperitoneally. This study was conducted over the 10-day period following irradiation (1000 rads cobalt-60) until the beginning of deaths due to radiation alone. These data were then associated with the status of tight junction barriers (zonula occludens) between epithelial cells of the ileum (Figure 1).

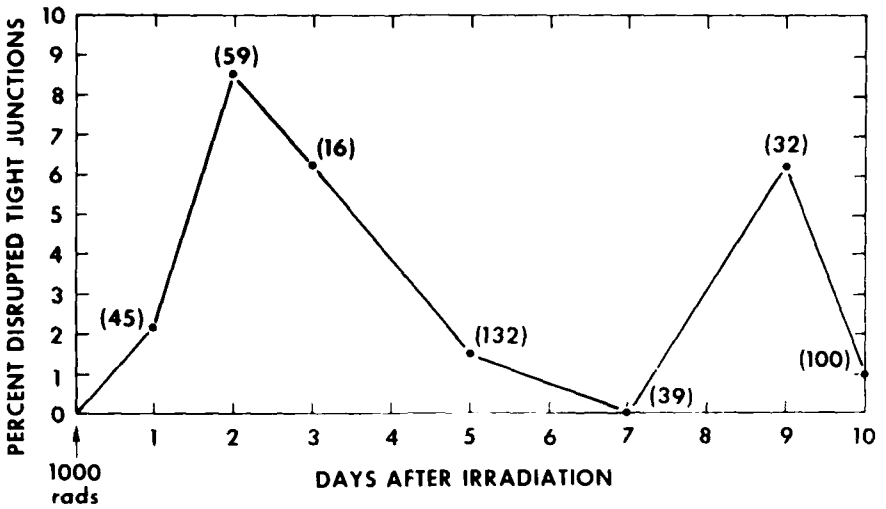
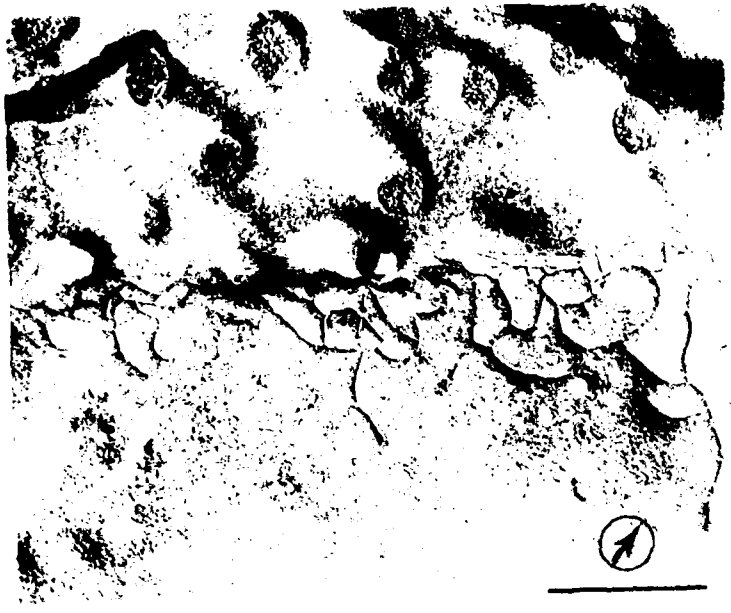


Figure 1. Incidence of detection of disrupted tight junctions in ilea of mice at various times after radiation. Number in parentheses by each point represents total number of tight junctional complexes observed at that time after irradiation. Ilea from four mice were used at each sampling time.

A biphasic pattern of sensitivity to endotoxin was observed in irradiated mice. Mice were resistant to endotoxin through day 2 after radiation, but sensitivity increased greatly (80% mortality) by day 3. Resistance increased by day 6 (6.7% mortality) and then dropped again by days 9 and 10 (100% mortality). Leukocyte numbers decreased 91% by day 2 (from 10,880 to 1,020 per mm^3) and further by day 3 (300 per mm^3). Leukopenia persisted for the duration of the experiment. Platelets began to decrease in number by day 5, and levels continued to drop until day 9. Disruption of some intestinal tight junctions was observed on days 1-5 after radiation (Figure 2). Junctional repair was evident by day 5 (Figure 3). Repair was noted as extensive junctional elements on ablumenal membrane fracture faces. A combination of leukopenia and leakage of endotoxin from the intestine may account for the increased sensitivity to endotoxin seen at day 3. Repair of the intestinal permeability barrier on day 5 coincided with reduced sensitivity to endotoxin. Later (7-10 days), bacteremia was present in host tissues, and mortality due to challenge with endotoxin again increased. Disruption of some tight junctional barriers was seen again on days 9 and 10 after radiation.



POSSIBLE ASSOCIATION OF GRANULOCYTE MOBILIZATION TO PERITONEAL CAVITY WITH ZINC CHLORIDE-INDUCED PROTECTION AGAINST ENDOTOXIN

Principal Investigators: R. I. Walker and S. L. Snyder, *AFRRI*
P. Z. Sobocinski, *U.S. Army Medical Research Institute of Infectious Diseases*
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We have attempted to determine which components of the inflammatory response are responsible for zinc chloride-induced retention of endotoxin in the peritoneal cavity and enhancement of survival following challenge with the toxin.

Zinc chloride injected intraperitoneally into mice caused accumulation of granulocytes (Table 1) in the peritoneal cavity, but these cells were apparently not responsible for the trapping process. This contention is supported by our observation that reduction of hepatosplenic uptake of chromium-51-labeled endotoxin in nonirradiated mice was similar (Table 2) to that in mice made leukopenic by irradiation (1000 rads cobalt-60) (1 rad = 10^{-2} J/kg). Hepatosplenic uptake was also depressed when nontreated mice were injected with endotoxin suspended in cell-free plasma. Furthermore, zinc did not protect irradiated mice challenged with endotoxin, although it enhanced survival in nonirradiated animals (Table 3). Lack of protection in irradiated mice may be due to a deficiency in cellular response in the peritoneal cavity.

Table 1. Alteration of Peritoneal Cell Oppulations[a] Induced by Zinc

Treatment,[b] 1 h	Total WBC,[c] cells mm^3	PMN,[d] %	Treatment,[b] 24 h	Total WBC, cells mm^3	PMN %
Saline-NT	2650 ± 461	21.5	Saline-NT	4750 ± 1838	11.2
Zinc-NT	4164 ± 640	56.4	Zinc-NT	7220 ± 2369	41.2
Saline-ET	1979 ± 709	8.4	Saline-ET	1306 ± 643	13.8
Zinc-ET	5416 ± 1920	46.8	Zinc-ET	Clump	69

[a] Six to 12 samples were counted in each group. Peritoneal washes from two mice were pooled to make each sample. Data for total WBC are presented as mean ± SD.

[b] Treatment $\xrightarrow{1 \text{ or } 24 \text{ h}}$ challenge $\xrightarrow{2 \text{ h}}$ sample collected. Saline- or zinc-treated mice received one of two treatments: ET = endotoxin (LD 0.8 mg) or saline (0.8 cm^3) at 1, 3, or 24 h later as indicated. Mice were sampled 2 h after the second treatment.

[c] White blood cells.

[d] Polymorphonuclear leukocytes.

Table 2. Hepatosplenic Uptake[a] of ^{51}Cr-Endotoxin Injected Intraperitoneally Into Saline or Zinc-Treated Mice

	% reduction of ^{51}Cr-ET in zinc group							
	Unirradiated				Irradiated			
	0	1 h	3 h	24 h	0	1 h	3 h	24 h
Spleen	84	82	70	83	82	71	74	82
Liver	44	79	77	48	57	89	55	82

[a] *Salmonella typhosa* endotoxin (0.25 cm^3) was administered i.p. at the indicated times postirradiation with zinc (0.4 mg) or saline (0.2 cm^3) and samples taken 2 h thereafter. Six to 12 mice were used in each group.

Table 3. Percentage of Mortality (48 h) in Irradiated [a] and Unirradiated Mice Treated With Zinc Chloride Prior to Challenge With Endotoxin [b]

	Unirradiated + endotoxin				Irradiated + endotoxin			
	Saline		$ZnCl_2$ [c]		Saline		$ZnCl_2$	
Treatment	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h
% mortality	57(14) [d]	100(20)	30(10)	20(20)	65(23)	75(12)	88(24)	75(12)

[a] Mice were irradiated with 1000 rad ^{60}Co at 45 rad/min.
[b] Irradiated mice were challenged i.p. with 0.2 mg Salmonella typhosa endotoxin; unirradiated animals received [illegible]
[c] Zinc chloride (0.4 mg/kg i.p.) was not lethal to irradiated or unirradiated mice when given without endotoxin.
[d] Number in parentheses refers to the number of animals in that experimental group.

RADIATION-INDUCED ALTERATIONS IN DISTRIBUTION OF LYSOSOMAL HYDROLASES IN RAT SPLEEN HOMOGENATES

Principal Investigators: S. L. Snyder and S. K. Eklund

Whole-body exposure to ionizing radiation can result in marked changes in the activities and/or distribution of lysosomal hydrolases found in lymphoid tissues. We postulated that lysosomal enzymes can act as inflammatory agents and therefore may contribute to stress and tissue injury in the irradiated animal. In support of this contention, we previously pointed out that the biphasic increases observed in serum and tissue lysosomal hydrolases after exposure occur concomitantly with a breakdown in normal "biological barriers" (1). In addition to their potential importance as inflammatory agents, lysosomal hydrolases may be useful as biologic markers for quantitating radiation-induced injury. Figure 1 shows the distribution of two lysosomal enzymes, beta-glucuronidase and alpha-fucosidase, in the spleen of irradiated rats as a function of dose 24 hours after exposure (2).

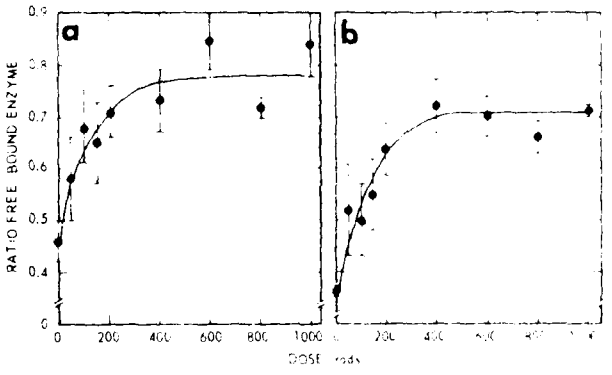

Figure 1. Effect of whole-body cobalt-60-gamma dose on ratio of free to bound beta-glucuronidase (curve a) and alpha-fucosidase (curve b). Error bars indicate standard error of mean.

These curves clearly indicate a large shift in activity of both enzymes from the particulate to the supernatant fractions at doses as low as 50 rads. Furthermore, the dose-response relationship for the ratio of free enzyme to bound enzyme suggests that it may be possible to use lysosomal enzymes as biologic indicators of radiation injury in an appropriate assay system.

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COLONY-FORMING CELLS IN THYMUS AND MESENTERIC LYMPH NODES OF MICE ENGRAFTED WITH LEWIS LUNG CARCINOMA CELLS

Principal Investigators: G. D. Ledney, T. J. MacVittie, D. A. Stewart, and G. A. Parker

In vitro culture of normal mouse thymus and mesenteric lymph node cells results in formation of colony-forming cells that are identified in part by their commitment to monocyte-macrophage cell development and their dependence on pregnant mouse uterine extract (PMUE) for growth. Behavior of these cells in the lymphoid organs of tumor cell-injected mice and their in vitro growth requirements may be important in understanding host-tumor relationships. This idea was tested by engrafting C57BL/6 mice with Lewis lung carcinoma cells and examining the thymus and the mesenteric lymph nodes for their colony-forming cell content at 3, 7, and 14 days after tumor-cell implantation. The cells were grown in PMUE only or in PMUE and normal human serum.

In cells obtained from Lewis lung cell-injected mice and grown in PMUE only (Figure 1), there was an approximate twofold increase in thymic colony-forming cells on day 3. A reduction to 50% of control thymic values was observed on day 7. The numbers of thymic colony-forming cells in control and tumor cell-injected mice were similar on day 14. The numbers of mesenteric lymph node colony-forming cells on day 3 increased twofold over control values and thereafter tended to be higher than controls.

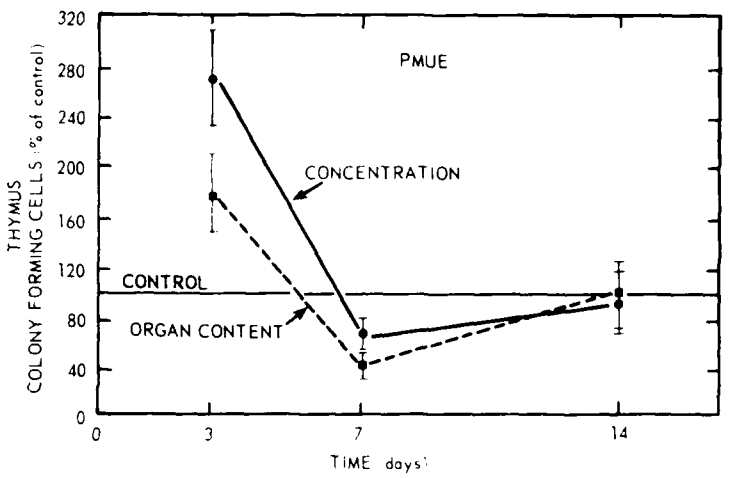

Figure 1. Percentage colony-forming cell (CFC) values of thymus cells obtained from mice injected with Lewis lung (3LL) carcinoma cells and grown in presence of pregnant mouse uterine extract (PMUE). Thymus cells were prepared from groups of mice at 3, 7, and 14 days after implantation with 3LL cells and grown under conditions described in Materials and Methods. No statistically significant differences in CFC values were obtained from mice injected with three quantities of 3LL cells. Thus each point represents mean value ± standard error of combined data of all three cell doses from six replicate experiments (n = 18). Thymic CFC values from 3LL-injected mice differed significantly from controls on days 3 and 7. Concentration (●—●) and organ content (■- - -■) of thymic CFC grown in PMUE and obtained from control mice were 19 ± 7 and 1540 ± 660, respectively. Control, ——.

With normal thymic and mesenteric lymph node cells grown in PMUE, maximum growth enhancement was noted by addition of 7.5% (volume for volume) normal human serum, which reduced the lag period by 30% for both types of cells, even though the maximum numbers of colonies were counted 25 days after culturing, as is the case for cells grown only in PMUE. The numbers of thymic colony-forming cells and node colony-forming cells grown in PMUE and normal human serum increased twofold and sevenfold, respectively, over that for cells grown in PMUE only.

Thymus cells obtained from tumor cell-injected mice and grown in PMUE and normal human serum (Figure 2) resulted in a twofold increase over controls of the thymic colony-forming cell concentration on days 3 and 7. By day 14, the thymic colony-forming cell values returned to control values. Lymph node cells grown in PMUE and normal human serum resulted in quantities of mesenteric lymph node colony-forming cells that did not differ significantly from control mice. However, the mesenteric lymph node colony-forming cell values of Lewis lung-injected mice tended to be lower than those for control mice on day 3.

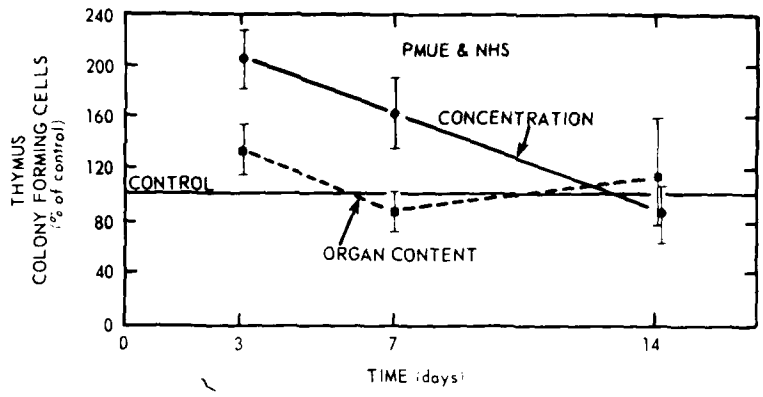

Figure 2. Description of this figure is same as Figure 1, except that concentration (●——●) and organ content (■--■) of thymic CFC grown in PMUE and normal human serum (NHS) and obtained from control mice were 25 ± 5 and 1560 ± 310, respectively.

In conclusion, the growth kinetics of thymic colony-forming cells and mesenteric lymph node colony-forming cells are profoundly altered by development of the Lewis lung carcinoma. The different numbers of colony-forming cells obtained after culturing in PMUE only or in PMUE and normal human serum could be interpreted as growth of two subpopulations of colony-forming cells.

IN VITRO AND *IN VIVO* ANALYSIS OF PRESERVED WHITE CELLS FOR POSTIRRADIATION TREATMENT

Principal Investigators: T. J. Contreras and J. F. Jemionek

Use of granulocyte transfusions as a beneficial adjunct in the therapy of gram-negative septicemia postirradiation has increased the need for large quantities of granulocytes for fresh transfusions or for preservation and later transfusion. Isolation of purified granulocytes, both human and canine, can be procured by the counterflow centrifugation-elutriation technique (Figure 1) from cells obtained from either peripheral blood (1,2) or continuous-flow centrifugation leukapheresis (CFCL) (3-5) (Table 1). This isolation has made the preservation of these phagocytic cells a more feasible objective.

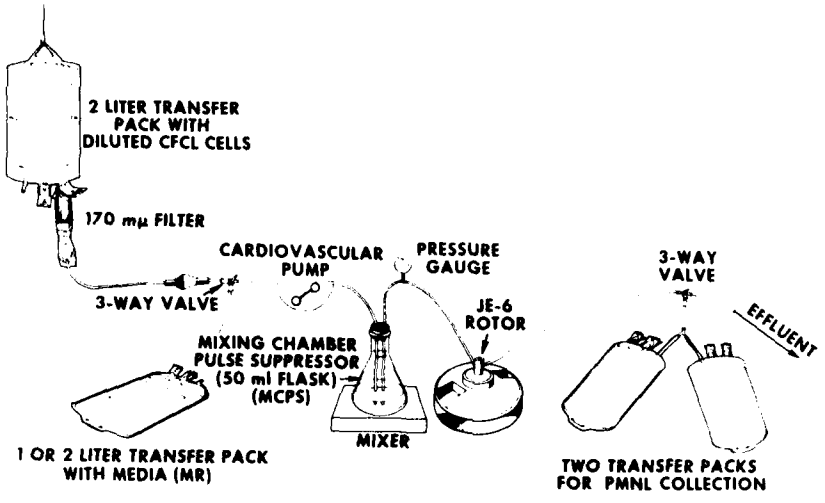

Figure 1. Modified dilution technique for isolation of granulocytes by counter-flow centrifugation-elutriation

Table 1. Granulocyte Purification of CFCL Concentrates by CCE

		CFCL CONCENTRATE				CCE ELUTRIATE			
		LEUKOCYTE DIFFERENTIAL			RBC/WBC	LEUKOCYTE DIFFERENTIAL			PMNL/RBC
		PMNL	MONO	LYMPH		PMNL	MONO	LYMPH	
GROUP A[a]	$\bar{x}$	62.3	10.3	27.6	40.6	98.9	0.9	0.2	50.7
$n = 10$	S.E.	2.5	2.0	1.9	5.9	0.2	0.2	0.1	7.0
GROUP B[b]	$\bar{x}$	52.8	15.8	31.4	41.1	95.1	3.7	1.2	67.8
$n = 12$	S.E.	1.5	2.3	1.2	4.4	1.3	1.1	0.4	9.7

[a] Studies to determine maximal capacity of elutriation chamber

[b] Studies to determine the efficiency of granulocyte recovery from CFCL concentrates

Differentials were estimated from air-dried smears stained in Wrights-Leishman stain. RBC/WBC and PMNL/RBC ratios were determined by electronic counts on a Coulter ZBI AND H4 Channelyzer

Our group preserved, in a liquid medium, canine granulocytes at 4°C for up to 15 days, with excellent results (6). Freeze preservation of canine granulocytes with 10% dimethylsulfoxide gave viable, functional cells with good overall morphology (7,8). In vivo evaluation of canine granulocytes isolated by continuous-flow centrifugation leukapheresis and counterflow centrifugation-elutriation is currently being done, using the beagle as an animal model. Preliminary results indicate that the dual isolation technique does not impair physiological function of the granulocytes.

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MEMBRANE POTENTIAL CHANGES DURING MACROPHAGE ACTIVATION

Principal Investigator: E. K. Gallin

Membrane potential changes were studied that occur during exposure of macrophages to chemotactic factors normally produced in response to infection after radiation.

Escherichia coli endotoxin-activated serum, added to macrophages, produced membrane hyperpolarizations associated with a decrease in membrane resistance (Figure 1). Fractionation of normal activated serum indicated that only fractions that eluted with a molecular weight of 12,500 produced membrane potential changes. The active material was identified as C5a (the small-molecular-weight cleavage product of C5) by heat stability and inactivation by goat antiserum to human C5 but not C3. Synthetic N-formyl methionyl peptides, which are chemotactic, produced similar membrane potential changes. These observations demonstrate that ion fluxes associated with membrane potential changes are early events in macrophage activation by chemotactic factors (1).

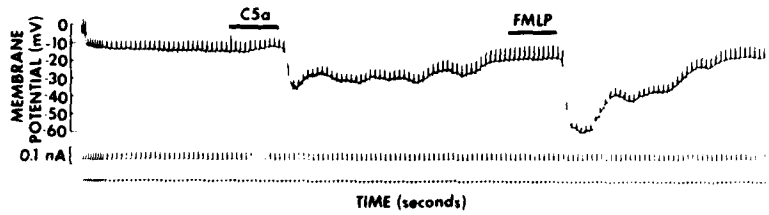

Figure 1. Response of macrophage to both C5a (5 μg/ml) and f-met-leu-phe (10^{-4} M) applied with blunt microelectrodes. Resting membrane potential = -10 mV.

These electrophysiological findings, together with other available data, were used to propose a model for the ionic events occurring during leukocyte chemotaxis (2). The model will be used to design experiments to activate and/or modulate leukocyte function so that, during times of stress (i.e., radiation injury), leukocyte activity can be enhanced.

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AN EXPERIMENTAL MODEL OF CHRONIC COMMUNICATING HYDROCEPHALUS

Principal Investigator:	W. J. Flor, *AFRRI*
Collaborators:	A. E. James, Jr., *Vanderbilt University Hospital, Nashville, Tennessee*
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Technical Assistance:	J. L. Parker and W. L. Sickel

Chronic communicating hydrocephalus (also known as normal-pressure hydrocephalus) is an adult form of hydrocephalus that can develop secondarily to head trauma, central nervous system infection, intracranial surgery, or spontaneous subarachnoid hemorrhage. The military population is as much at risk to these cere[illegible]ts as the civilian population, and often more so. The disease process is a [illegible]ssively debilitating one in which the ventricles within the brain (which contain cerebrospinal fluid) expand at the expense of the surrounding neural tissue. Current surgical treatment techniques are unsatisfactory, and pathological mechanisms of disease progression are incompletely characterized.

Physiological studies (1,2) of the animal models we developed implied that compensatory mechanisms for the reabsorption of cerebrospinal fluid are established in the hydrocephalic animals. Our physiological data strongly suggested that cerebrospinal fluid reabsorptive pathways are established across the ependymal cell layer between the brain ventricles and brain tissue.

We recently presented corroborative anatomical evidence for the establishment of transependymal reabsorption pathways. In studies (3) in animals by both transmission and scanning electron microscopy, the areas of most severe pathology along the dorsolateral angles of the lateral ventricles have been identified and characterized at various times after disease induction (100-1000 days). In animals from the 100-day postinjection group, severe denudation of the ependymal cell covering of the dorsolateral angles was accompanied by traceable channels into the brain parenchyma and by proliferation of small, round supraependymal cells. By 1000 days, the ventricular surface in this region of most severe pathology had developed a glial/ependymal "scar" covering much of the previously denuded surface. Through this scar, patent fluid channels into periventricular brain tissue could still be traced. Fluid following these pathways would destroy subependymal brain substance, resulting in increasing ventricular size and chronic deterioration of the clinical condition of the experimental animal (or the patient).

In a collateral study (4), we determined that enlargment of the distal canal of the spinal cord does not play a significant role in the developing pathophysiology of communicating hydrocephalus (as opposed to obstructive hydrocephalus).

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TESTS OF BIOLOGICAL INTEGRITY IN DOGS EXPOSED TO AN ELECTROMAGNETIC PULSE ENVIRONMENT

Principal Investigator: S. J. Baum

Dogs were exposed to an electromagnetic pulse (EMP) environment for 8 hours each day for 45 days. At the end of that time, they had received 5.8×10^6 EMP at 5 pulses/sec with a peak electric field intensity of 447 kV/m.

Biological tests were conducted to ascertain concentration of erythrocytes, leukocytes, neutrophils, lymphocytes, reticulocytes, and platelets. Bone marrow samples were obtained by biopsy from the ribs 7 days before and 7 days after the last EMP exposure for assessment of mitotic rubricytes and myelocytes.

Pregnant female dogs were exposed to the EMP environment in order to study possible gross effects on fetuses. Reproductive capabilities of irradiated male animals were tested for 1 year after the last exposure. None of these tests revealed any injury in the EMP-irradiated dogs.

NEUROBIOLOGY DEPARTMENT

The Neurobiology Department is tasked with the study of mechanisms whereby ionizing radiation influences the cellular components of the nervous system. The Department is organized into three Divisions, each focusing on separate types of preparation and techniques for study of nervous tissue. The Radiation Biophysics Division uses biophysical and electrophysiological approaches to the study of elementary mechanisms underlying nerve cell activity, the communication between cells, and the alteration of these normal processes by ionizing radiation. Most of the studies performed in this Division use isolated nervous tissues obtained from invertebrates, lower vertebrates, or mammals, in which one is able to more completely control the environment and variables than in the intact animal. The Cellular Neurobiology Division uses primarily tissue-cultured neurons, glia, and muscle cells in order to achieve the aim of availability of a homogeneous population of cells of mammalian origin that can also be maintained in a controlled environment. Finally, the Neurological Sciences Division uses intact animals, including primates, to study directly the effects of ionizing radiation on nervous system function.

The effects of radiation on nervous tissue that concerned us principally during this fiscal year are those after relatively high doses. At doses of 800 rads and greater, some primates exhibit the syndrome known as early transient incapacitation (ETI). ETI is a transient decrement of performance of the animal, often occurring with a 5-min latency after exposure to radiation and lasting for a period of a few minutes to an hour. ETI is frequently accompanied by a dramatic fall in blood pressure. The origin of this syndrome is not well understood, although previous work in our laboratories (as well as the laboratories of others) suggests that the syndrome results from release after radiation of an active humoral agent that acts at specific receptor sites on blood vessels and probably on neurons to alter the animal's ability to respond. Much of the Department's research is aimed at identifying these substances released after radiation and elucidating their sites of action as well as the mechanisms whereby they alter the level of consciousness and ability to respond. We are studying this problem in each Division with a variety of techniques and preparations. Previous work showed that a likely site of origin of the active materials is the mast cell. Mast cells store large quantities of biogenic amines–particularly histamine, serotonin, and dopamine–and also some active peptide factors. Ionizing radiation at relatively low doses is known to cause mast cells to release these materials. One of our research programs involves study of the mast cells directly. Other programs involve investigation of the effect of the substances released from mast cells on smooth muscle, glia, and nerve cells. Receptors for histamine, serotonin, and dopamine are widespread, and unfortunately we do not know whether the primary site of action is on the vasculature or directly on neurons. One of our most significant advances in this regard, however, has been the progressive understanding that a variety of responses can be elicited from any single active agent. We have found that histamine, serotonin, dopamine, and a variety of other peptides and neurotransmitter substances can be either excitatory or inhibitory, depending on the nature of the postsynaptic receptor. We still do not know if ETI results primarily from interaction of these agents with vascular or neuronal receptors, but it is possible and perhaps likely that the syndrome results from activation from multiple receptors at multiple sites. These studies are proceeding in invertebrate neurons, in cells in tissue culture, and in the intact central nervous system of mammals and primates.

Another major effect of ionizing radiation on the central nervous system is one that occurs only at quite high doses, called the central nervous system syndrome. This syndrome is the cause of death in animals exposed to greater than 1000 rads. It is characterized by progressive depression of the central nervous system, with alterations in behavior and visceral function, terminating in coma and death. The molecular events causing this syndrome are poorly understood, but previous studies in our laboratory implicated radiation-induced alterations of intracellular calcium as one causative factor. Intracellular calcium regulates a great variety of membrane processes including membrane permeability, exocytosis of neurotransmitters and hormones, and probably levels of cyclic nucleotides and other biochemical components of the cell. Several of our research programs are involved in study of the roles of calcium in these various systems.

In a nuclear detonation, the energy is released in three principal forms: radiation, thermal energy, and energy in the form of blast and overpressure. Effects on a human in the vicinity result from each factor alone or in combination. Several studies in the Neurological Sciences Division concern investigation of the effects of mechanical trauma on central nervous system function and the interaction of mechanical and radiation injury. One proposal studies the effects of thermal injury, especially on visual functions. Particularly in the central nervous system, injuries from quite different insults frequently are manifest in the same ways: cerebral edema, alterations in cerebral blood flow, extravasation of blood into the intracranial space, or selective loss of neurons or glial cells at the site of injury. Consequently, the programs studying different aspects of injury to central nervous system tissue are closely interrelated. Many of the techniques and procedures for these studies are identical, and often conclusions obtained in one experiment are applicable to studies of a different form of injury. Several other sites of injury are of interest to the Department, including effects of radiation on the cardiovascular system and the auditory and vestibular systems.

VOLTAGE-DEPENDENT EXCITATORY RESPONSE TO SEROTONIN IN *APLYSIA*

Principal Investigators: J. C. Pellmar and D. O. Carpenter

A slow, voltage-dependent excitatory response to iontophoretic application of serotonin has been observed in voltage-clamped neurons of Aplysia californica. The response has a time to peak of 15-30 sec and a duration of 1-3 min. At potentials more negative than about -40 mV, the response is absent. As the cell is depolarized, the serotonin-evoked inward current becomes progressively larger. The potential dependence of the serotonin response is similar to that of delayed rectification. The response is accompanied by an apparent decrease in conductance, which can be attributed either to a decrease in conductance to potassium or to a regenerative inward current. Changes in extracellular potassium concentration up to 30 mM have little effect, but higher concentrations reduce the amplitude of the response. The actions of zero-sodium solutions depend on the sodium substitute: glucosamine prolongs the response to serotonin whereas sucrose and mannitol greatly reduce the amplitude. Exposure to lithium-substituted seawater causes a gradual attenuation, and subsequent replacement of normal seawater potentiates the response. The serotonin response is minimally affected by alterations in extracellular calcium but is blocked by cobalt and manganese (Figure 1). Observed changes in response amplitude are usually accompanied by consistent changes in delayed rectification.

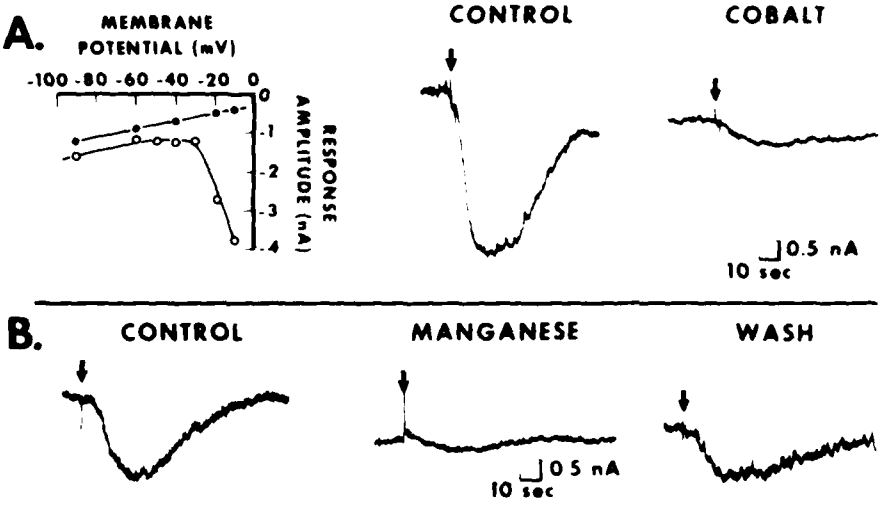

Figure 1. Actions of calcium blockers cobalt and manganese. *A* shows that 25 mM cobalt in calcium-free seawater blocks component 2 (inward current at potentials more depolarized than -30 mV) but minimally affects component 1 (inward current at more hyperpolarized potentials). Current traces in *A* are from same cell and show responses to serotonin when membrane potential was voltage-clamped to -10 mV in normal seawater (control) and in presence of cobalt. Inward current is represented by downward deflection. *B* shows current response to serotonin in another cell when membrane potential was clamped to -14 mV while in normal seawater (control), in 25 mM manganese, in normal seawater, and following replacement of normal seawater (wash). ○, control; ●, 25 mM cobalt.

It is difficult to determine the ionic basis of the serotonin response from these data. A regenerative inward sodium current can be ruled out, but such a current carried by calcium remains a possibility. Alterations of extracellular calcium may not alter the calcium gradient sufficiently to modify such current. The above data seem most consistent with a decrease in potassium conductance, yet it is disturbing that moderate changes in potassium concentration have minimal effects.

AFFERENTS TO MEDIAL PONTINE RETICULAR FORMATION AS DEMONSTRATED BY RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE

Principal Investigator R. W. Greene, *WRRI*
Collaborator M. Schnilder, *WRRI*
Associate Investigator G. B. Stanton, *Howard University*

Giant neurons of the brain stem are known to be involved in movement, and other important functions have been proposed. They may have a function in sleep states of consciousness. They may be an important locus of action for action substances that are released by ionizing radiation and that mediate early transient incapacitation. In order to better understand the function of these cells, we have attempted to study the afferent input to this area using anatomical techniques.

Figure 1 shows the labeled cells that project to the reticularis pontis caudalis (RPC). Injection of the RPC and the abducens nucleus produced labeled cells bilaterally in the deep and intermediate layers of the superior colliculus but none in the superficial layers. Labeled neurons in the contralateral superior colliculus were most numerous in the rostral half (12 large and 14 medium-sized neurons/60-μm section). Moreover, labeling intensity of the large cells was greater in the medial third of the superior colliculus, suggesting that these soma have the largest number of axon terminals within the injection site. On the ipsilateral side, labeled neurons were few and faintly labeled. No projections from the superior colliculus to the abducens nucleus were seen following horseradish peroxidase studies by Maciewicz et al. (1). Furthermore, our control injections resulted in relatively few labeled cells in superior colliculus, confirming the localization of terminals of superior colliculus-RPC projection to the caudal pole of the RPC.

Another major projection to the RPC was from the vestibular nuclei. Cells were most numerous in the ipsilateral medial, inferior, and ventrolateral vestibular nuclei, but were also seen in these nuclei on the contralateral side. Other nuclei labeled by injection in the RPC include, on the ipsilateral side, the nucleus of the field of Forel, the nucleus of Darkshevich, the interstitial nucleus of Cajal, the mesencephalic reticular formation, and the trigeminal spinal nucleus. Nuclei labeled bilaterally include intermediate-sized cells in the caudal part of the facial nucleus, the pontine raphe nuclei, the pontine lateral tegmental fields, the RPC, and the reticularis gigantocellularis.

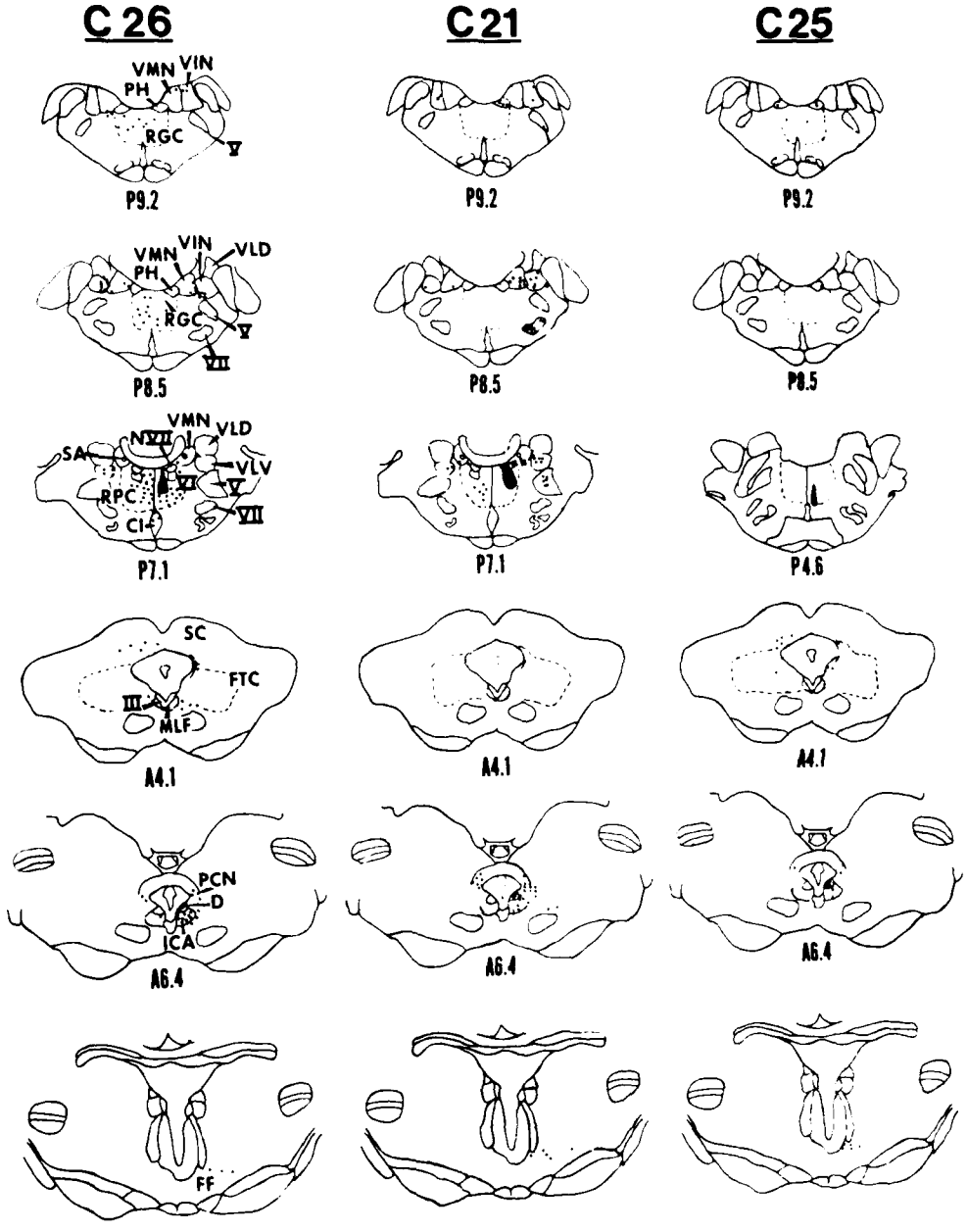

Figure 1. Location of labeled cells (● = one labeled cell) for medial injection (C26), lateral injection (C21), and rostral injection (C25). Area of horseradish peroxidase spread from injection is marked in black for each case. PH, perihypoglossal nucleus; VMN, medial vestibular nucleus; VIN, inferior vestibular nucleus; RGC, reticularis gigantocellularis; V, trigeminal spinal nucleus; VLD, dorsolateral vestibular nucleus; VII, facial nucleus; NVII, genu of the facial nerve; VLV, ventrolateral vestibular nucleus; RPC, reticularis pontis caudalis; VI, abducens nucleus; CI, inferior central; SC, superior colliculus; FTC, frontal tegmental field; III, oculomotor nucleus; MLF, medial longitudinal fasciculus; PCN, nucleus of the posterior commissure; D, nucleus of Darkshevich; ICA, interstitial nucleus of Cajal; FF, nucleus of field of Forel.

These results demonstrate that the cells of origin of the superior colliculus-RPC projection are the large and intermediate-sized neurons in the deep and intermediate layers of the superior colliculus.

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ROLE OF CALCIUM IN SECRETORY PROCESSES RELATED TO EARLY TRANSIENT INCAPACITATION

Principal Investigator: M. A. Donlon, *AFRRI*
Collaborators: G. N. Catravas, *AFRRI*
M. Kaliner, *National Institute of Allergy and Infectious Diseases, NIH*

Since 1949, histamine release following midlethal doses of X radiation has been reported in rats, hamsters, and man. The primary site of histamine storage resides in the tissue mast cell, but the mechanism of radiation-induced histamine release is unknown. It is known that external calcium is required for activation of the secretory mechanism in mast cells, and that the entry of calcium into the cell is sufficient stimulus to cause histamine release (1).

This study was undertaken to investigate calcium transport and histamine release in highly purified (>95%) preparations of rat peritoneal mast cells (RPMC). These preliminary studies will be followed by identical experiments on X-irradiated cells to determine if radiation-induced alterations in calcium influx serve as stimulus for histamine secretion.

The kinetics of calcium association with purified RPMC's is presented in Figure 1. Mast cells were incubated at 37°C in a spinner flask containing Hepes-buffered salt solution, 0.8 mM calcium, 1.4 μCi/ml cobalt-45, and 7.5 μCi/ml ^3H_2O (the last used as an indicator to monitor cell volume). Samples were removed from the flask and centrifuged through silicone oil. All radioactivity not associated with the cell pellet remained in the supernatant, which was also analyzed for histamine release. The oil layer was suctioned off, the results were dissolved, and the radioactivity determined. Mast cell-associated calcium rapidly increased and reached a plateau by 1-2 min, followed by a slow and continuous accumulation. Cell volume remained constant during the experiment, and minimal histamine release occurred.

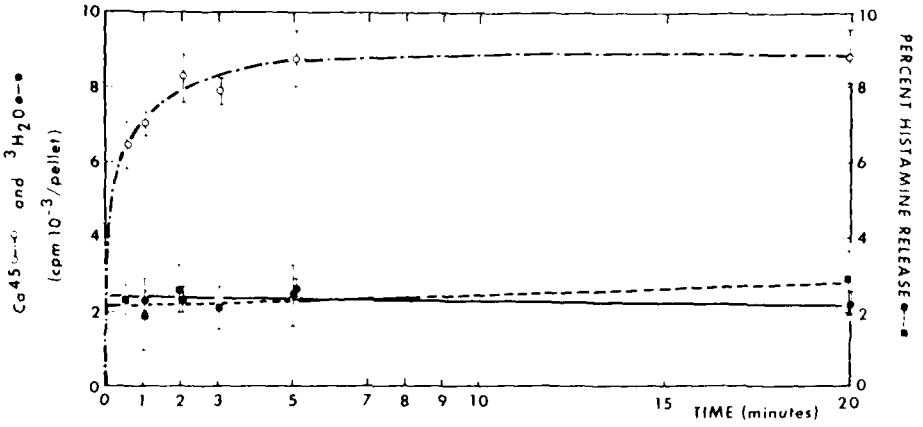

Figure 1. Kinetics of cell-associated radioactivity and histamine release in highly purified nonstimulated rat peritoneal mast cells. Each point represents mean of triplicate samples ± S.E.

It was necessary to distinguish between the externally bound (superficial) calcium and the transported (intracellular) calcium. This was accomplished by incubating previously labeled mast cells with the impermeant chelator ethanedioxy-bis-(ethylamine)-tetraacetic acid (EGTA). The results are shown in Figure 2. It is seen that about 70% of the total cell-associated calcium resided in an EGTA-displaceable pool while the remaining fraction resided in an internal compartment. This is the first demonstration of two calcium pools in the isolated nonstimulated cell. Further studies on the dynamics of these pools during stimulation and secretion are now in progress.

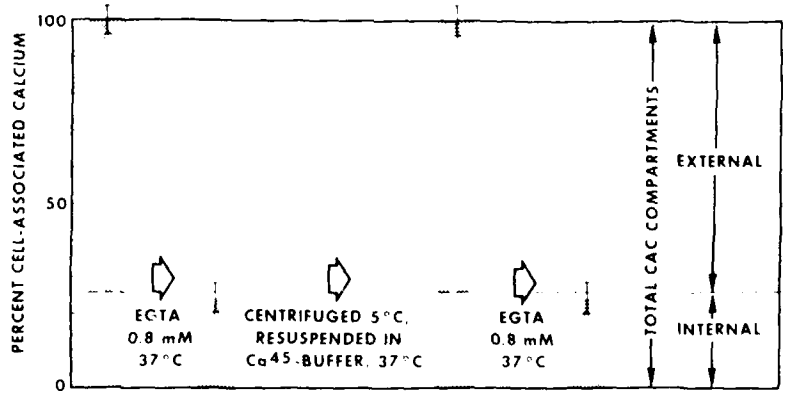

Figure 2. Identification of two cellular calcium compartments in nonstimulated mast cells. EGTA treatment of RPMC reveals an EGTA-sensitive (external) and an EGTA-insensitive (internal) cell-associated calcium (CAC) compartment.

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VISCOSITY CHARACTERISTICS OF MIDDLE EAR EFFUSIONS

Principal Investigator: M. L. Wiederhold, *AFRRI*
Associate Investigators: J. T. Zajtchuk, J. G. Vap, and H. O. deFries, *Uniformed Services University of the Health Sciences, Bethesda, Maryland*

It is commonly assumed that effusions present in the middle ear for a short duration are thin and produce a mild hearing loss, whereas effusions present for a longer duration become more viscous with time and produce a greater hearing loss. In order to test this hypothesis, we used cats as an experimental model. The eustachian tube was ligated on one side to produce middle ear effusions, and hearing loss was measured electrocochleographically. At various times after eustachian tube ligation, tympanocentesis was performed, and physical properties of the effusion were measured.

After eustachian tube ligation, all cats developed negative middle ear pressure within 3 days and flat (Type B) tympanograms within 7 days. Hearing loss also developed early and became maximal at about 3 weeks. The initial tympanocentesis varied from 6 days to more than 300 days after eustachian tube ligation. Thin fluids were found in five cats at 13-168 days after ligation. Glue was obtained in six other cats at 6-136 days after ligation. In the remaining four cats, not enough fluid could be obtained to characterize. Within a few days after tympanocentesis, the cat's eardrum resealed, as evidenced by observation and tympanometry. This allowed for repeated sampling of effusions. These data indicate that a significant difference does not exist between the amount of hearing loss associated with thin fluids and with glue. Viscosity is not clearly related to duration of fluid in the middle ear. A positive correlation does seem to exist between hearing loss and specific gravity of the fluid.

MEMBRANE VOLTAGE NOISE ASSOCIATED WITH CILIARY BEATING IN THE *APLYSIA* STATOCYST

Principal Investigator: M. L. Wiederhold

The Aplysia statocyst is a spherical balance organ. The wall of the cyst consists mainly of 13 receptor cells with motile cilia projecting into the fluid-filled cyst lumen. Dense statoconia settle to fill the bottom third of the cyst lumen but are kept in continual random motion by the beating cilia. A receptor cell is excited when the preparation is tilted sufficiently to bring its cilia into contact with the statoconia. The excitation consists of a depolarization and an increase in membrane voltage noise (Figure 1).

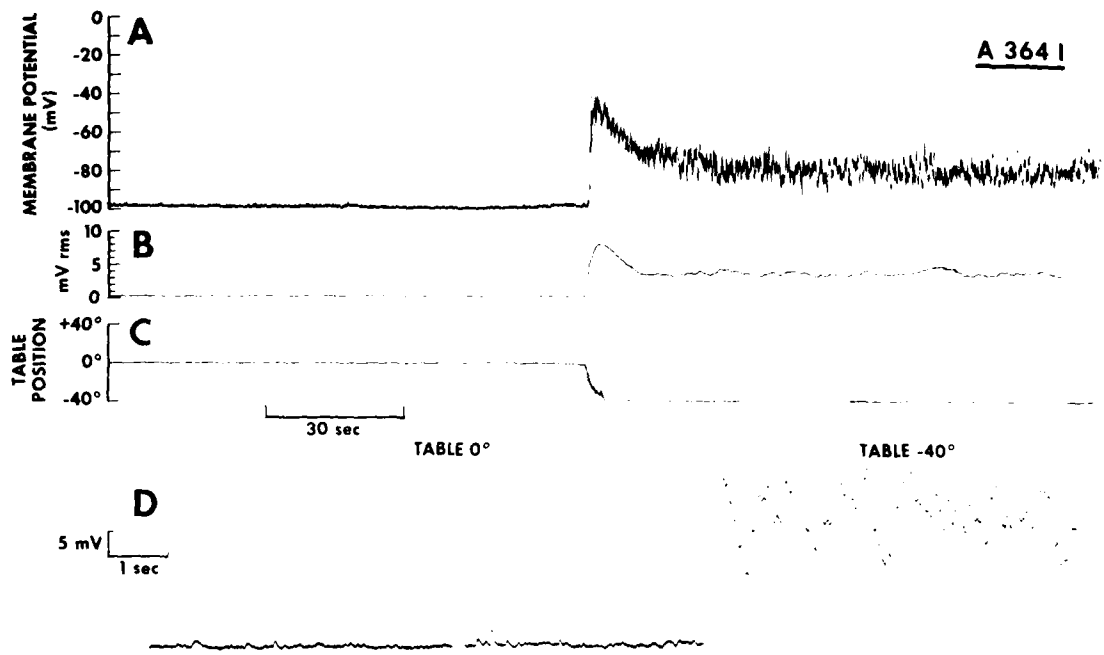

Figure 1. Response to tilting a preparation in normal artificial seawater. *A*. Membrane potential as table is lowered from level position (0°) to cell-down position (-40°). Spike amplitude is distorted by pen recorder. Actual spikes are 100 mV peak to peak. Tilting indicated in trace *C*. *B*. Root mean square membrane potential measured with an AC-DC converter, passing frequencies from 0.16 Hz to 110 kHz. Due to settling time to converter, readings are accurate for only 25 sec after sudden change in membrane potential. *D*. Portions of trace *A* with scales expanded. Note discrete positive potentials from 0.5 to 3.5 mV amplitude.

Analysis of the membrane noise caused by excitation is presented, which indicates that the noise is a superposition of many discrete depolarizing events whose average amplitude is 1.5-2.3 mV. These are interpreted as arising from collisions between the moving statoconia and the beating cilia. With a receptor cell positioned so that only occasional collisions between the statoconia and cilia would occur, isolated discrete events of the size predicted by the analysis are seen. The conductance change associated with these events is about 10 times larger than estimates of single ionic channel conductance from other studies.

When a preparation is treated with seawater containing 10 mM nickel chloride (used in other studies to paralyze the cilia of paramecium), the motility of these cilia is reduced or blocked, and both the depolarizing and noise-increase responses to tilting are either greatly reduced or abolished. Another agent that blocks ciliary motility is a factor in the serum of cystic fibrosis patients. When such serum was mixed with seawater, results similar to those with nickel chloride treatment were obtained. The mechanisms by which the ciliary motility might contribute to the sensory function of these cells are discussed elsewhere (1).

Since hair cell receptors in the mammalian inner ear can be affected directly by ionizing radiation, this model system will allow us to study the physiological basis of such radiation effects at the membrane level.

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AUDITORY RESPONSES IN CATS PRODUCED BY PULSED ULTRASOUND

Principal Investigators: K. R. Foster and M. L. Wiederhold

Auditory nerve responses and cochlear microphonics are produced in cats by pulsed 5-MHz ultrasonic energy from a transducer placed against the dura mater. The pulses must be relatively intense ($\simeq$30 W/cm^2) to produce a response but can be sufficiently brief (less than 70 μsec) to not observably heat the brain tissue.

The cats apparently respond to radiation pressure transients accompanying the absorption of ultrasound in the brain tissue. Both amplitude and latency of the N_1 neural responses to the ultrasound can be matched to those produced by relatively weak tone pips or clicks from an external source (Figure 1). The cochlear microphonic produced by a pulse shows prominent ringing at 5-10 kHz in different cats. Amplitude of the N_1 response exhibits broad maximum, for constant amplitude pulses, at pulse widths of 20-60 μsec. This variation of the N_1 response amplitude with pulse width is similar to that of a high-pass filter with a cutoff frequency at the dominant frequency of the cochlear microphonic, which is tentatively identified with a ringing frequency of the skull.

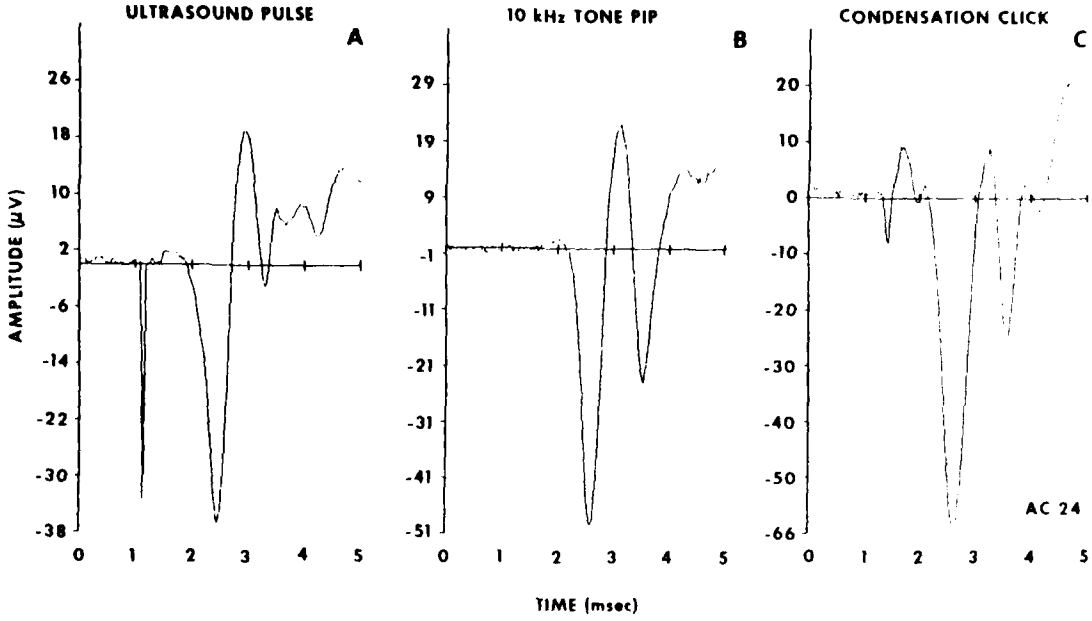

Figure 1. Computer-averaged responses to ultrasonic stimulation and to 10-kHz tone pips and 0.1-msec condensation clicks. *A*. Ultrasound pulse (50 μsec, 5 MHz, 33 W/cm^2); 500 responses. *B*. Tone pip (1 msec, 10 kHz, 42 dB re 0.0002 dyn/cm^2 rms) of random phase, short rise, and full times; 200 responses. *C*. Condensation clicks (0.1 msec, 65 dB peak pressure); 200 responses. All stimuli were repeated at 10 sec^{-1}. Cat AC24

HEARING LOSS AFTER EUSTACHIAN TUBE LIGATION MEASURED ELECTROCOCHLEOGRAPHICALLY

Principal Investigator: M. L. Wiederhold
Associate Investigators: S. A. Martinez, M. G. Pierson, and H. O. deFries
Technical Assistance: D. M. Paull and R. E. C. Scott

A system is described that allows repeated, noninvasive recording of auditory nerve responses in the cat to transient acoustic stimuli using a closed acoustic system (1). N_1 responses to clicks are recorded from a stainless steel ring electrode at the end of a hollow earbar, the tapered end of which is made of insulating plastic. Acoustic stimuli are generated by a dynamic earphone coupled to the earbar. A calibrated probe microphone is also incorporated into the earbar to measure sound pressure near the tympanic membrane. This allows better stimulus control than is available with free-field systems. To facilitate insertion of the earbar, meatoplasties were performed on all animals. Responses recorded with this system in anesthetized cats are described and compared to those recorded at the round window (see Figure 1). Good

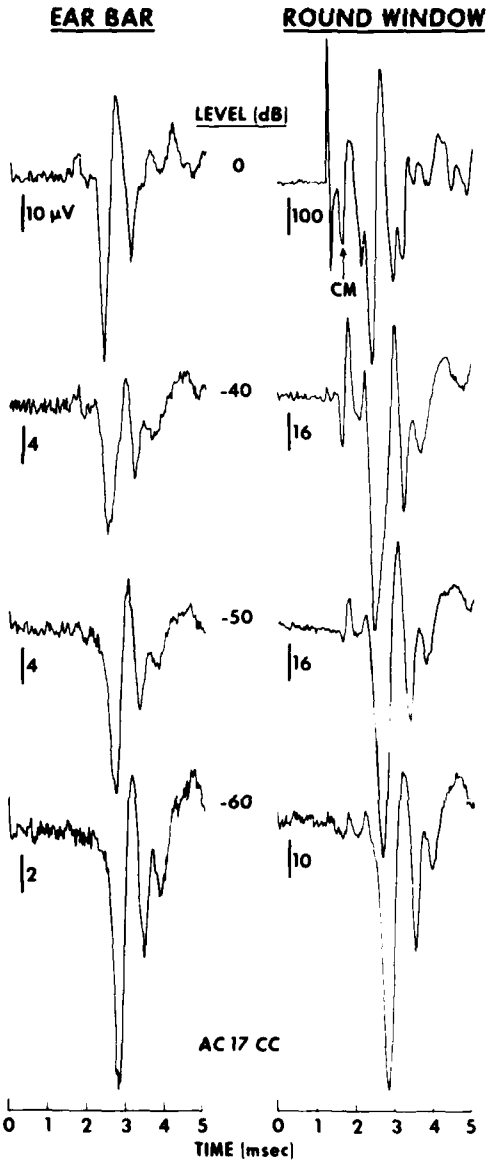

Figure 1. Comparison of averaged responses from ear-bar electrode (left column) and wire electrode 1 mm posteroventral to round window in same acute cat (right column). Responses shown for 0, -40, -50, and -60 dB for 128 dB sound pressure level peak pressure condensation clicks. All averages are of 256 responses.

repeatability of measurements is described for an animal population of 20 domestic cats over a period of several months. For some of these animals, response amplitude varied from one session to another, but response latency, especially for condensation clicks, was consistent. By comparing statistics of multiple measurement of both N_1 amplitude and latency for rarefaction and condensation clicks, it is concluded that the N_1 latency-versus-click level function for condensation clicks provides the most reliable measure of the cat's auditory nerve function.

Auditory nerve responses to condensation and rarefaction clicks were recorded from the external ear canal of cats using a closed acoustic system. Repeated control recordings from both ears formed a baseline for each of four animals used in this study. After a baseline had been established, the eustachian tube on one side was ligated and serial recordings of N_1 responses were performed for up to 140 days postligation. By comparing the shift that occurred in the N_1 latency-versus-click level plots after ligation, the equivalent hearing loss was determined (2). In all cases where the eustachian tube was successfully ligated, the loss was progressive for the first 20 days and then usually showed some transitory improvement. The loss stabilized after 60 days, varying from 15 to 40 dB in different animals (see Figure 2). In addition to N_1 recordings, serial tympanograms were also measured. These indicated negative middle ear pressure in the first 2 days postligation and the presence of middle ear fluid by 1 week postligation.

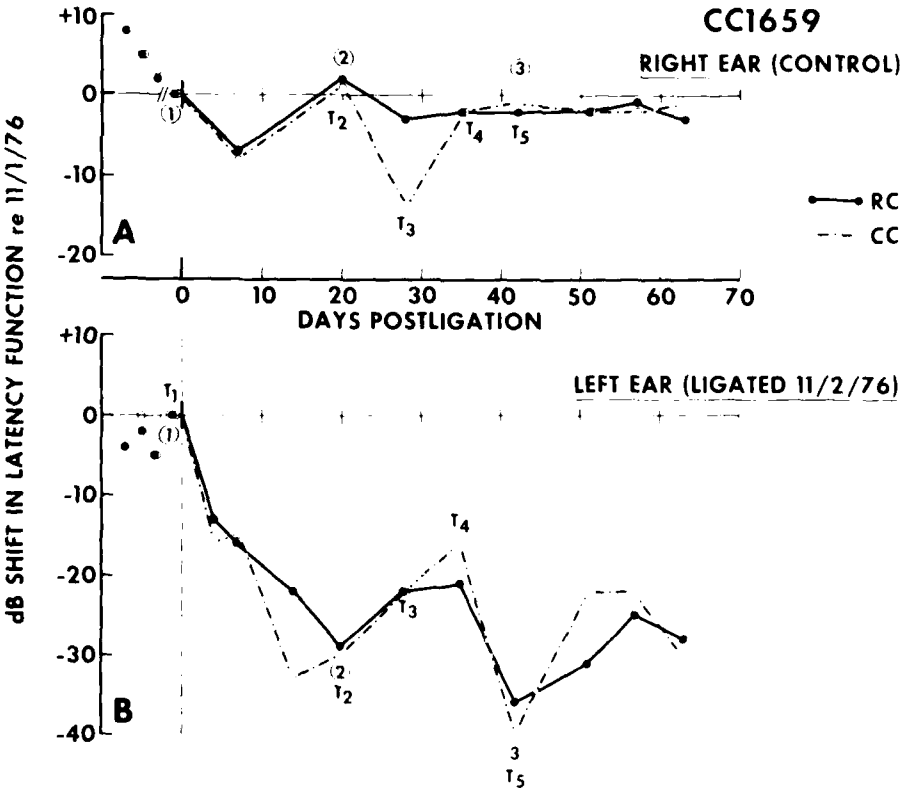

Figure 2. Shift in latency functions (hearing loss) for rarefaction (RC) and condensation clicks (CC) after ligation of the left eustachian tube of cat CC1659. Shifts are all relative to preoperative control latency functions (points labeled 1 for each ear). Points to left of day 0 are earlier preoperative controls.

Since the development of middle ear fluid is a common complication of radiation exposure to the head, this system will allow us to assess the effects on hearing of different levels of radiation exposure.

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DEXAMETHASONE AND EXPERIMENTAL RADIATION NECROSIS OF THE BRAIN. I. EFFECT OF EARLY TREATMENT

Principal Investigators: A. N. Martins, *Walter Reed Army Medical Center*
R. E. Severance, *AFRRI*
J. M. Henry, *Armed Forces Institute of Pathology*
T. F. Doyle, *AFRRI*

Delayed radiation necrosis of the brain is a dreaded, often debilitating, and occasionally fatal complication of therapeutic irradiation of the brain. The pathogenesis of delayed radiation necrosis of the brain remains a subject of continuing controversy. The most widely held view is that most if not all of the lesions ultimately develop as the direct consequence of injury to blood vessels. By injuring the genome, ionizing radiation presumably prevents replication of endothelial cells. The affected cells die after a variable latency, setting the stage for progressive failure of the microcirculation and leading to vasogenic edema from a break of the blood-brain barrier, petechial hemorrhages, and thrombosis. Secondarily, the parenchyma manifests a broad spectrum of ischemic changes from reactive gliosis to areas of frank infarction.

A wide variety of drugs are often used in association with radiotherapy, and these may affect the resistance of brain tissue to ionizing radiation. In particular, the glucocorticoids are frequently given in large pharmacologic doses to patients being irradiated in order to reduce brain edema and intracranial pressure. Some of its salutary effect presumably derives from stabilizing the cell membranes of capillary endothelium and parenchymal cells, which reduces brain edema and loss of cellular potassium. It has also been suggested that glucocorticoid protects the central nervous system from free radical attack. Confronted with the facts that both glucocorticoid and ionizing radiation affect DNA synthesis as well as the permeability of capillary membranes, we questioned whether they act synergistically or antagonistically on the brain.

Eighteen juvenile, male, rhesus monkeys (Macaca mulatta) were assigned in equal numbers at random to either an experimental or a control group. The experimental group received dexamethasone and the control group received saline. Each monkey of the experimental group received 4 mg of dexamethasone intramuscularly twice daily, morning and night, for 12 days. Thereafter, over the subsequent 12 days, the daily dose of dexamethasone was gradually reduced to zero and then permanently discontinued. Animals of the control group received an equal volume of saline.

Irradiations were carried out on the second day of dexamethasone administration, between 1/2 and 2 hours after the third dose had been given. A standard primate chair was modified to hold a collimator and lead body shield. In order to irradiate the entire brain of an alert animal, a collimator was used that also served as a restraint for the head. The irradiations were performed with a linear accelerator at approximately 20 MeV with a beam current and pulse width adjusted so that the total dose of 1800 rads was delivered to the whole brain in 8.5 min ± SD. After irradiation, the animals were returned to their cages. They were observed and examined until they died or until terminal inanition required that they be sacrificed.

Eventually all animals developed a neurological syndrome characterized by anorexia, marked behavioral changes, and motor impairment (Table 1). Most striking, when it developed, was an inexorably progressive loss of coordination and strength of all four extremities, usually coupled with disequilibrium and a hunched-over posture. Individual animals of both groups developed unique neurologic signs. Some manifested byoclonic jerks of all muscle groups in response to sudden tactile or auditory stimuli. Others developed torticollis and retrocollis, truncal dystonia, and circus movements. The rate of progression of the neurologic syndrome varied from animal to animal and ranged between 5 to 68 days from time of first sign of death. There was no statistically significant difference between the two groups regarding weight change, latency of onset of neurologic syndrome, rate of progression of neurologic syndrome, or length of survival following irradiation (Table 1). The external surface of the brains of all monkeys from both groups appeared normal. There was no statistically significant difference in mean weight of the brains or in gross morphology of the brains between the two groups.

Table 1. Effects of 1800 Rads Radiation Delivered in 8.5 Minutes to Whole Brain of Two Groups of Rhesus Monkeys

	Saline-Treated (n = 9)	Dexamethasone-Treated (n = 9)	p
Onset of epilation	* 38 ± 13.1	* 24 ± 7.8	0.05
Onset of behavioral change	106 ± 31.7	108 ± 31.9	0.91
Onset of motor impairment	109 ± 23.0	109 ± 24.4	1.0
Duration of neurologic syndrome	27 ± 10.7	30 ± 21.5	0.71
Survival time	137 ± 30.5	139 ± 36.9	0.87

* in days, mean ± SD

Microscopic appearance of the radiation-induced lesions tended to be monotonously stereotyped. We observed no statistically significant difference in histological appearance, number, or distribution of radiation-induced lesions between the dexamethasone and control groups. Dexamethasone failed to alter susceptibility of the primate brain to radiation-induced delayed necrosis. These results suggest that the common clinical practice of using glucocorticoids together with therapeutic radiation in the treatment of brain neoplasms does not increase the risk of developing delayed radiation necrosis. Unfortunately, the results also provide no support for the view that glucocorticoids can protect normal brain from the deleterious effect of therapeutic radiation.

RESPONSE OF CEREBRAL CIRCULATION TO TOPICAL HISTAMINE

Principal Investigators: A. N. Martins, *Walter Reed Army Medical Center*
T. F. Doyle and S. J. Wright, Jr., *AFRRI*

Due to continuing uncertainty about the preponderant effect of histamine on brain blood flow (1,2), we undertook an in vivo study to determine local response of cerebral circulation of the cat and the monkey to topically applied histamine.

Small bilateral parietal craniectomies were made in cats and in cynomolgus monkeys anesthetized with ketamine and nitrous oxide. The dura was opened, and polarographic electrodes of thin platinum wire were inserted into the cortex of each hemisphere. Mock cerebrospinal fluid was irrigated continuously onto the brain surrounding the electrodes from which local cerebral blood flow was determined repeatedly by hydrogen clearance. The pial vascular bed was observed at 40X magnification throughout the experiment and photographed for documentation. After stable baseline cerebral blood flow was established, solutions of histamine in mock cerebrospinal fluid (in various concentrations ranging from 10^{-5} M to 10^{-2} M) were irrigated onto one hemisphere; the opposite hemisphere served as control. Histamine consistently dilated vessels on the surface of the brain in both species, and produced within 15 min a dose-related local hyperemia of the brain that subsided 30-60 min after histamine was removed (Tables 1,2).

Table 1. Effect of Topical Histamine on Local Cerebral Blood Flow in the Cat

		Histamine Concentration (M)			
	Baseline	10^{-5}	10^{-4}	10^{-3}	10^{-2}
Cerebral blood flow*	61 ± 5	65 ± 8	113 ± 20	122 ± 11	124 ± 27
Number of animals	17	8	11	16	5
p†		0.048	0.0002	0.000005	0.004

* ml/100 g/min ± S.E.

† Probability that difference from baseline value would have occurred by chance (paired t test)

Table 2. Effects of Topical Histamine on Local Cerebral Blood Flow* in Cynomolgus Monkey

		Histamine Concentration (M)		
	Baseline	10^{-5}	10^{-4}	10^{-3}
Monkey No. 1	73	69	111	148
2	47	46	53	65
3	104		156	
4	53			93

* ml/100 g/min

These results imply that histamine is capable of participating in the acute response of the microcirculation of the mammalian brain to physiologic and pathologic stimuli.

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BIODYNAMICS AND PATHOPHYSIOLOGY OF TRAUMATIC UNCONSCIOUSNESS

Principal Investigators: E. N. Gunby, W. A. Flor, S. A. Oliva, and T. F. Doyle
Technical Assistance: R. E. Severance and V. A. Kieffer

A series of 40 experiments were conducted to evaluate the relationship of changes produced in cortical evoked responses by both mechanical trauma and radiation exposure. The HAD-III device was used to create an acceleration-deceleration injury in 26 macaque monkeys. This device simulates exposure to bomb blast as might be seen in a nuclear warfare environment. Unconsciousness resulted in subjects in a number of experiments, and the cortical evoked responses were monitored. We found that the disappearance and recovery of these responses correlated with severity of the injury and responsiveness of the animal. It is thus possible to determine a threshold of decelerative force required to produce unconsciousness with or without recovery.

Using this model of brain dysfunction due to mechanical trauma, we then performed an independent series of 14 experiments designed to simulate early transient incapacitation due to radiation exposure. Macaques were exposed to 7500 rads of whole-body irradiation using the AFRRI linear accelerator. Changes were observed in the cortical evoked responses that correlated with apparent physical incapacitation in about two thirds of the experiments. Later injection of histamine failed to produce similar changes in evoked responses. Thus it appears that the cortical evoked response may offer a method of quantitating radiation incapacitation and investigating the mechanism of this phenomenon. Within the limits of cerebral blood flow autoregulation, histamine did not produce the changes in cortical evoked responses that were seen with radiation exposure alone.

EFFECT OF CARDIAC PACEMAKER ISCHEMIA ON CARDIAC FUNCTION IN THE RHESUS MONKEY

Principal Investigators	W. A. A[illegible] III and R. N. Haw[illegible], AFRRI
Collaborators	S. P[illegible], St. Michael's College, Winooski, Vermont
	D. F[illegible], United States Air Force Academy, Colorado Springs, Colorado
Technical Assistance	V. A. K[illegible] and L. Parkhurst

Exposure of rhesus monkeys to supralethal doses of ionizing radiation results in a dramatic decline in systemic arterial pressure (1). Deficits in tissue blood flow in critical organs (brain, heart, kidney) during this period of decreased perfusion pressure may contribute to the performance decrement, which has also been observed in this animal model (1). In an effort to delineate the effects on cardiac function of a blood flow deficit to the cardiac pacemaker (sinus node), rhesus monkeys were studied during occlusion of the artery that perfuses the sinus node region of the right atrium.

Sinus node artery occlusion for up to 60 min resulted in the onset of a decline in heart rate within 8 $\pm$ 2 sec. This was usually accompanied by a shift in the pacemaker site to either an ectopic atrial or atrioventricular junctional site with several instances of atrial arrhythmias. An example of atrial fibrillation during pacemaker ischemia is shown in Figure 1. Five minutes after the onset of occlusion, several episodes of chaotic atrial activity were recorded. These arrhythmias led to a decreased ventricular contractile force and, as a consequence, a decline in systemic blood pressure. After these episodes, a stable junctional pacemaker emerged and persisted for the duration of the occlusion period.

To determine involvement of the autonomic nervous system in these events, propranolol (0.5 mg/kg) and atropine (0.5 mg/kg) were administered and then the sinus node artery occlusions were repeated. Ischemia in the sinus node region still elicited a decline in heart rate, but the overall decrease (-17 $\pm$ 4 beats/min) was less than that observed during the first occlusion period (-65 $\pm$ 12 beats/min). This difference probably reflects the lower baseline heart rate after autonomic blockade. The incidence of arrhythmias was also markedly reduced.

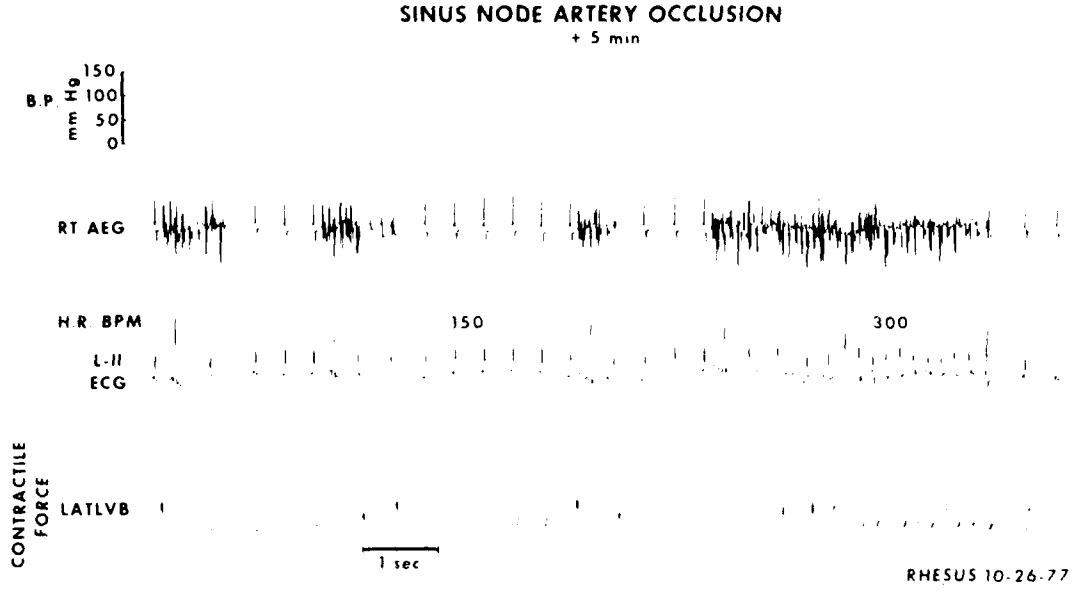

Figure 1. Effect of sinus node artery occlusion on cardiac function. B.P., blood pressure; RT AEG, right atrial electrical activity; H.R. BPM, heart rate in beats per minute; L-II ECG, lead-II electrocardiogram; LATLVB, contractile force recorded from strain gauge attached to lateral wall of left ventricle.

Based on these results, it is concluded that localized ischemia within the sinus node region leads to bradycardia and arrhythmias, both of which will adversely affect cardiac function. It appears that ischemic periods of up to 1 hour still allow recovery of sinus node pacemaker function. This is in contrast to the irreversible damage that occurs in ischemic ventricular myocardium within about 20 min of coronary artery occlusion (2).

Radiation-induced hypotension may lead to myocardial underperfusion. Based on results of the present study, it appears that ischemia in the sinus node may occur but that stable pacemaker function can reemerge if blood flow levels are restored within 1 hour.

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ACUTE CHANGES IN CARDIAC RESERVE OF CATS AFTER EXPOSURE TO A SUPRALETHAL DOSE OF RADIATION

Principal Investigators: R. N. Hawkins and W. A. Alter III
Technical Assistance: V. A. Kieffer

In the rhesus monkey, the cardiovascular events following a lethal dose of ionizing radiation seem to be a transient fall in arterial pressure and an accompanying increase in cardiac output (1,2). After a variable period of time, however, these parameters recover to values within normal physiological ranges before a total and fatal collapse of the circulatory system. The transient fall in arterial pressure has been attributed to peripheral circulatory failure whereas the increased cardiac output has been said to result from the reflex compensatory effect by the heart in response to depressed arterial pressure. These projects restricted their analysis to cardiovascular function in the unstressed animal. It is conceivable that even more dramatic alterations in function will be seen when the cardiovascular system is stressed by increased demands during performance of physical tasks.

This study was undertaken to determine the maintenance of cardiac reserve after radiation. It is this cardiac reserve that is used whenever the heart must increase its output in response to increases in venous return. Chloralose-anesthetized cats were used in the experiments completed during this fiscal year. Animals were exposed to 10,000 rads of 14.5-MeV electrons at a dose rate of 13,000 rads per minute. This dose failed to produce significant changes in resting levels of arterial pressure and heart rate. However, within minutes, maximum cardiac work [cardiac output X (arterial pressure - right atrial pressure)] declined, reaching a minimum value at 30 min postirradiation. At this time, maximum cardiac work was only 60% of control. It then showed a trend toward recovery and was at 90% of control by 90 min postirradiation.

Heretofore, the myocardium has been considered relatively radioresistant, but based on these results, it appears that there is an acute change in mechanical function of the heart. Blood samples were taken at 3 hours postirradiation to determine the presence of circulating cardiodepressant humoral agents that may have contributed to this decline in cardiac work. A cardiodepressant agent has been found in the blood of cats, and levels of this agent are reported to be increased during periods of depressed cardiac function (3). Results of the samples drawn from the irradiated cats are not available at the end of this fiscal year.

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BEHAVIORAL AND PHYSIOLOGICAL CHANGES IN THE CAT AFTER A SUPRALETHAL DOSE OF RADIATION

Principal Investigators: W. A. Alter III and R. N. Hawkins, *AFRRI*
Collaborators: S. Poulin and J. Phillips, *St. Michael's College, Winooski, Vermont*
J. Hill, *United States Air Force Academy, Colorado*
Technical Assistance: V. A. Kieffer

Occurrence of an acute performance decrement in man after high doses of radiation has not been documented, and the number of cases (n < 12) is too small to allow any conclusions. In lieu of data from man, study of the patterns of behavioral and physiological changes in several animal species after irradiation may provide evidence on the likelihood of similar changes in man.

Early performance decrement after irradiation has been observed in the monkey (1) and pig (2) whereas the dog (3) has shown no evidence of an early performance decrement under similar conditions of radiation exposure. The present study was undertaken to study acute responses of the cat to 10,000 rads of 14.5-MeV electron radiation. Behavioral effects were investigated in conscious cats, and cardiovascular function was studied in chloralose-anesthetized cats.

Results to date indicate that conscious, unrestrained cats experience a dramatic pupillary constriction (miosis) within minutes of radiation exposure. These animals became lethargic, but, if aroused, they demonstrated intact motor reflexes with some gait abnormalities related to hindlimb incoordination. In addition, these animals frequently had difficulty negotiating around obstacles, which was probably related to visual limitations resulting from miosis. Emesis and diarrhea were also evident within the first 2 hours. Most of these neurologic and gastrointestinal symptoms persisted for the 3-hour observation period, and there was inconsistent recovery of the pupils to preexposure diameters. In anesthetized cats, radiation failed to alter resting levels of systemic arterial pressure and heart rate.

Based on these results, it is concluded that the cat does experience acute neurologic and behavioral changes after exposure to this supralethal dose of radiation. The effect of miosis, lethargy, and mild ataxia on the animal's performance of a cognitive or physical task has not been evaluated, but these symptoms most likely indicate a diffuse change in function of the central nervous system during the immediate post-exposure period. It appears that this radiation dose did not produce significant changes in basal cardiac rate or systemic blood pressure. These data, however, do not permit conclusions on ability of the cardiovascular system to respond to the stresses of physical activity required to perform a motor task.

Further studies are under way to reveal the etiology of the miosis, acute gastrointestinal symptoms, and ataxia.

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EFFECT OF LOW-SODIUM SOLUTIONS ON CONDUCTANCE IN THE GIANT ABDOMINAL NEURON OF *APLYSIA*

Principal Investigators: J. P. Apland and D. R. Livengood

Low-sodium external solutions have been reported by various authors to cause an increase, a decrease, or no change in conductance of molluscan neurons (1). A careful investigation was undertaken to establish whether a conductance change could be demonstrated consistently in the giant abdominal neuron and, if so, to establish the ionic mechanism involved. Numerous sodium substitutes were used, including Tris, mannitol, magnesium-mannitol, glucosamine, tetraethanolammonium, tetramethylammonium, bis-(2-hydroxyethyl)-dimethylammonium, choline, and arginine. Ramp-generated current-voltage plots were used to establish slope conductances. A conductance increase was consistently observed with most of the substitutes. This change could be blocked by extracellular application of 30 mM cobalt chloride (Figure 1).

These results imply that calcium is involved in the phenomenon. In some experiments no conductance change was observed in low-sodium solutions. In these experiments a conductance decrease could be demonstrated following application of cobalt. This suggests that conductance increase, which could be blocked by cobalt, was masked by a decreased sodium conductance brought about by removal of external sodium. It is not yet clear whether the conductance increase in low-sodium solutions is due to a direct increase in calcium conductance or to a calcium-mediated increase in potassium conductance. It was also observed that application of cobalt abolished anomalous rectification in these cells. This suggests that anomalous rectification, which is dependent on external potassium, may involve a calcium-mediated increase in potassium conductance. Conductance changes and alterations of electrogenic pumps are involved in radiation effects on neuronal membranes.

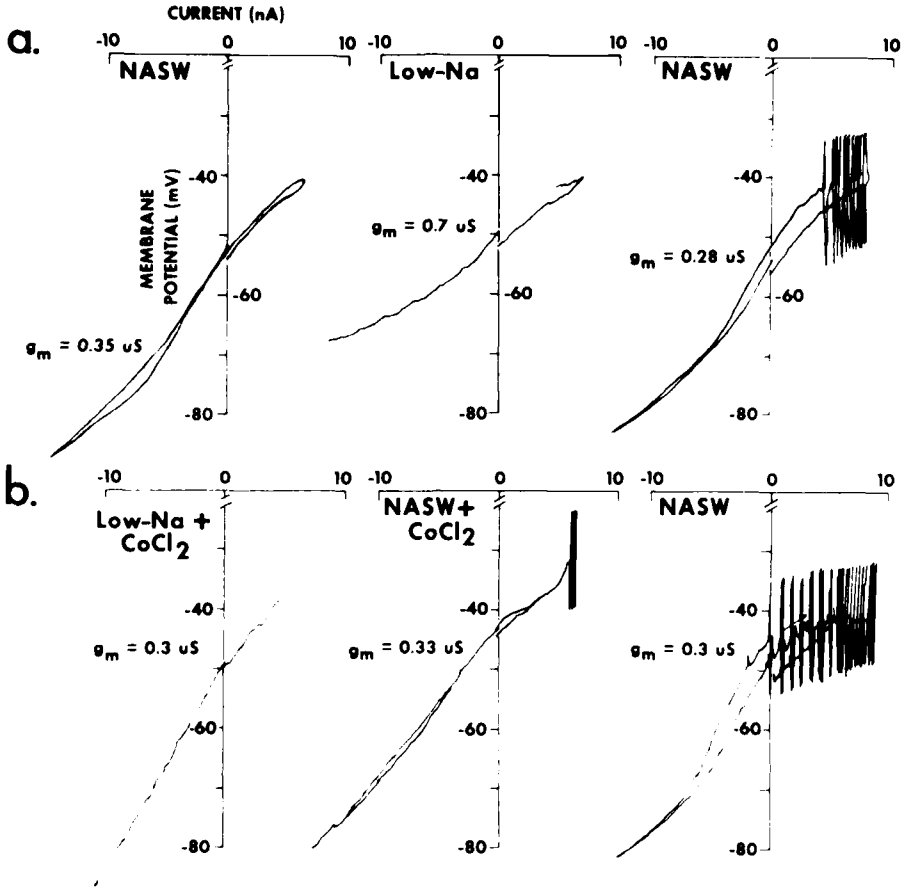

Figure 1. Blockade by cobalt of increase in g_m induced by low-sodium seawater. NASW, normal artificial seawater. Low-sodium seawater was prepared by substituting glucosamine for 90% of sodium. Current-injecting microelectrode became plugged during production of current-voltage relations in both low-sodium and low-sodium + cobalt solutions, but single complete excursions were obtained in both hyperpolarizing and depolarizing directions. *a*, Current-voltage relations demonstrate an increase in g_m in low-sodium seawater, with complete recovery on return to NASW. *b*, Current-voltage relations for same cell showing g_m nearly identical in NASW and in low-sodium seawater after addition of 30 mM cobalt to both solutions. Hyperpolarizing limb of current-voltage relation also became linear (anomalous rectification was abolished) after application of cobalt. Original shape of current-voltage relation was restored after washout of cobalt.

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THE TRIPLE HELIX: A POSSIBLE STRUCTURE FOR EXCITABLE MEMBRANE CHANNELS

Principal Investigator: H. R. Guy

Although excitable membrane channels differ in ion selectivities and gating mechanisms, some drugs (e.g., local anesthetics, barbiturates, and strychnine) apparently block a large number of these channels. This suggests that the structures of the ion-conducting portions of these channels are similar. I have explored the possibility that this structure is a beta helix.

Beta helices are perhaps the simplest family of peptide structures that form ion-permeable channels in membranes. Of many beta helices that I have analyzed, a triple-stranded left-handed beta helix with slightly more than 15 residues per turn ($^{3L}\beta^{15}$ helix) is most consistent with electrophysiological and pharmacological data obtained from five biological channels: the sodium and potassium channels underlying the action potential, the fast inhibitory postsynaptic potential (ipsp) chloride channels and excitatory postsynaptic potential (epsp) sodium channels of Aplysia neurons, and the end plate channels of frog skeletal muscle. The polypeptide (Ser-Gly-Apl-Gly-Apl)$_n$, where Apl is a residue with an apolar side chain, should form a $^{3L}\beta^{15}$ channel (see Figure 1) which has a high conductance, is relatively nonselective, and is blocked by drugs that block all five of the biological channels. Differences between the ion selectivities of the biological channels may be due to a more ion-selective segment of a $^{3L}\beta^{15}$ helix, which is in series with this nonselective segment. Sequences that form selectivity filters for the respective channels consistent with experimental data are: (Ser-Ala-Pro-Ala-Pro) for end plate, (Asp-Ala-Pro-Ala-Pro) for action potential sodium, (Arg-Ala-Pro-Ala-Pro) for ipsp chloride, and (Gln-Ala-Pro-Gly-Apl) for action potential potassium. Some drugs that affect only one channel type can bind at the selectivity filter. These include tetrodotoxin and saxitoxin for the action potential sodium channel; tetraethylammonium and its derivative or the action potential potassium channel; and pentylenetetrazol, penicillin G, bicuculline, and picrotoxin for the ipsp channel. The nonselective conformation should be able to undergo a conformation change to a closed state. Large molecules that enter the open channel should inhibit this conformational change. A number of molecules (e.g., batrachotoxin, aconitine, grayanotoxin, veratridine, and DTT) may prolong the open conformation of action potential sodium channels by only partially blocking the nonselective segment of the channel while preventing its closing. This theory can be tested by synthesizing the polypeptides, incorporating them into membranes, and comparing the pharmacology and ion selectivities of the synthetic channels to those of biological channels. Synthetic channels that are similar to biological channels should be ideal for analyzing the molecular mechanisms by which radiation affects membrane channels.

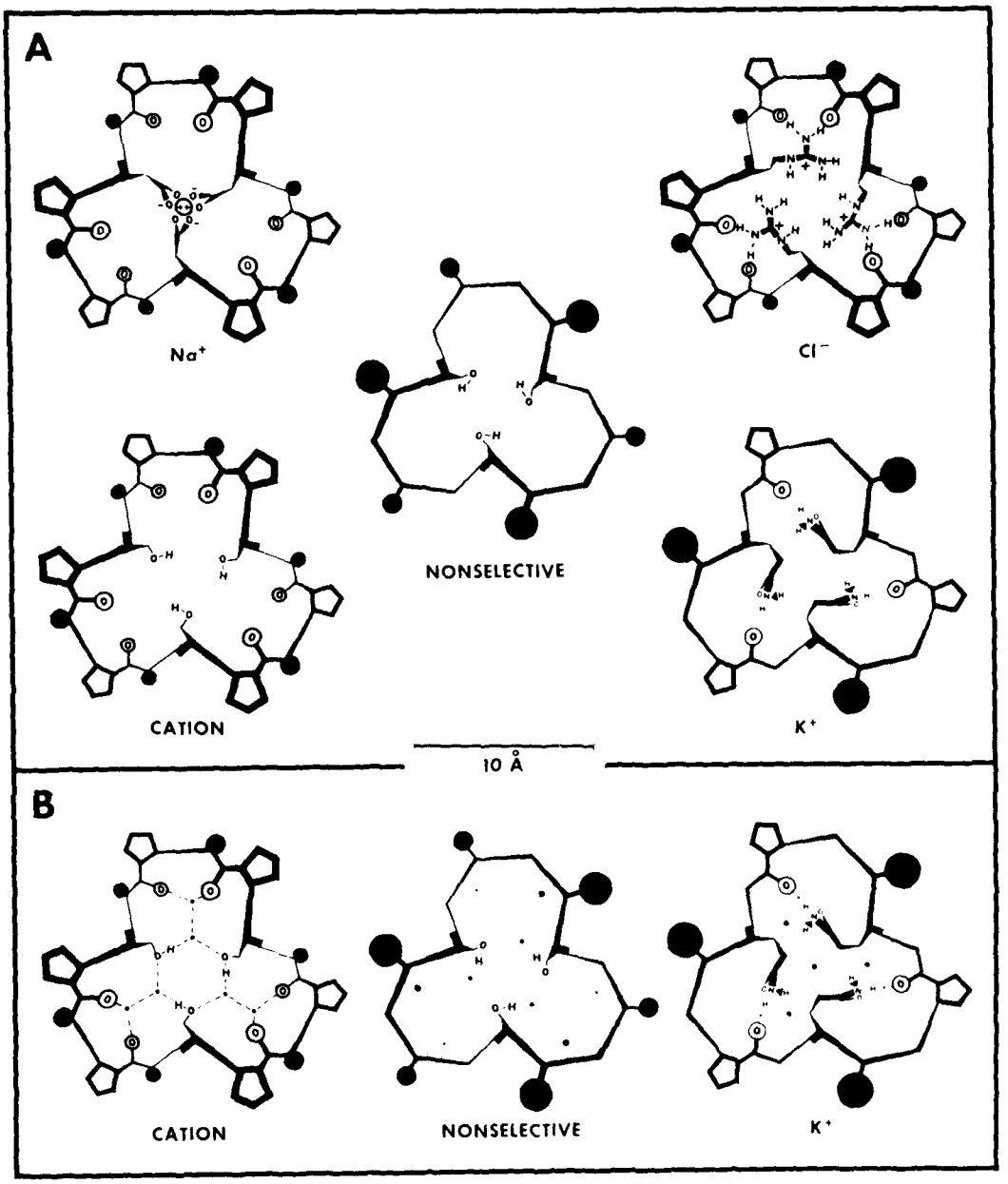

Figure 1. Schematic representation of $^{3}L_{\beta}{}^{15}$ helix. Heavy lines represent peptide backbone, darkened circles are hydrophobic side chains, pentagons are Pro side chains, and unfilled circles are carbonyl oxygens of peptide bond between Ala and Pro. Sequences are: nonselective (Ser, Gly, npl, Gly, npl)$_n$; Na^+ (Asp, Ala, Pro, Ala, Pro), Cl^- (Arg, Ala, Pro, Ala, Pro); cation (Ser, Ala, Pro, Ala, Pro), and K^+ (Glu, Ala, Pro, Gly, npl)$_n$. *B* shows how water H (hydrogen) binds in cation, nonselective, and K^+ channels. Center of water molecules is represented by solid dots and H bonds by dashed lines. Water molecules above plane of figure are represented by larger dots, and those below plane by smaller dots. Length and angles of H bonds are approximately those of ice.

BLOCKADE OF NEUROMUSCULAR TRANSMISSION BY ENZYMATICALLY ACTIVE AND INACTIVE β-BUNGAROTOXIN

Principal Investigators: D. R. Livengood, M. A. Donlon, L. M. Masukawa, G. S. Tobias, and W. Shain, *AFRRI*
Collaborator: R. S. Manalis, *University of Cincinnati*

β-Bungarotoxins have been shown to be presynaptic blockers of neuromuscular transmission. Experiments are reported using the most positively charged β-bungarotoxin that elutes from a CM-Sephadex C-25 column. The toxin is known to be a single polypeptide with a molecular weight of about 11,000, and it has phospholipase A_2 activity. Application of the enzymatically active toxin to the frog sciatic nerve-sartorius muscle preparation results in initial decrease in the average endplate potential amplitude followed by a temporary rebound in endplate potential amplitude, and finally complete inhibition of endplate potentials. Similarly, miniature endplate potential frequency is initially reduced after toxin application but then increases dramatically. After phospholipase A_2 of the toxin is inactivated, treatment with the toxin results in only the initial decrease in transmitter release (Figure 1).

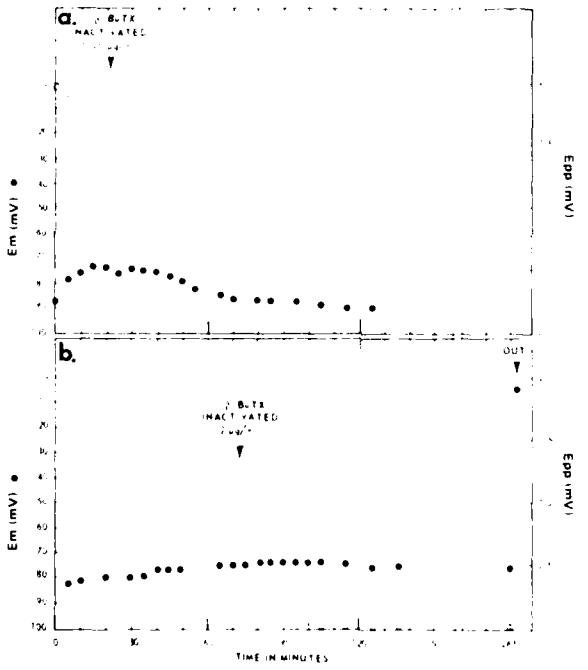

Figure 1. Blockade of end plate potential (epp) by addition of enzymatically inactive beta-bungarotoxin (β-BuTX). Amplitude of epp (o) is indicated by right ordinate, and membrane potential (●) is indicated by left ordinate. Purified β-BuTX was added to bath (arrow) with final concentration as shown. *a*. Incomplete blockade of epp was produced by addition of inactivated toxin to 0.05 μg/ml (final concentration). *b*. Complete blockade of epp was produced by addition of 2 μg/ml (final concentration) (first arrow). Washout of toxin was started 30 min after toxin application, but epp showed no indication of recovery by time recording electrode was withdrawn 2 hours and 45 min after start of washout period. Experiments shown in *a* and *b* are from different preparations.

These results suggest that this β-bungarotoxin acts in two functionally separate steps: (a) by binding to a specific presynaptic site possibly associated with calcium entry, and (b) by perturbing the presynaptic membrane by its enzymatic action, which results in increase and then failure of transmitter release.

The action of β-bungarotoxin is very similar to the effect of radiation on the neuromuscular junction. Therefore, the inactivated toxin may give us an important biological probe for the study of radiation-related effects on neuromuscular junctions.

DISPARITY BETWEEN NEURONAL FIRING AND DEOXYGLUCOSE UPTAKE IN INFRARED SYSTEM OF PIT VIPERS

Principal Investigators: C. R. Auker, R. M. Meszler, and D. O. Carpenter

^{14}C-2-deoxyglucose accumulates in areas of the central nervous system that are metabolically active, but the cellular functions that require this metabolic energy are uncertain. In the infrared-sensing system of pit vipers, we found an area that does not accumulate ^{14}C-2-deoxyglucose despite a stimulus-induced increase in single-unit firing rate.

In rattlesnakes, both eyes and one pit or one eye and both pits were excised. One group of snakes were stimulated with a heat lamp strobed at either 2 Hz (250 msec duration) or 0.1 Hz (1 sec duration) while single-unit recordings were made from the contralateral optic tectum. In pit-intact animals, both frequencies increased single-unit activities of some units deep in the tectum. After the first few pulses, rapid adaptation decreased the response to the high-frequency stimulation but not the low-frequency stimulation. A second group of snakes were injected intracutaneously with 50 μCi of ^{14}C-2-deoxyglucose and stimulated for 75 min at either of the above frequencies.

Brains were processed for deoxyglucose radiography. No labeling of the optic tectum was seen in any of the pit-intact animals, but dense labeling of the superficial tectum was seen in eye-intact animals. Gainer and Schwartz (personal communication) proposed that the primary energy-requiring function of neurons is sodium transport. Thus, small neuronal processes and terminals with high surface/volume ratios would have the highest energy requirements and would preferentially accumulate ^{14}C-2-deoxyglucose. The lack of ^{14}C-2-deoxyglucose uptake by the infrared system in the optic tectum may result from low synaptic input density. Consistent with this hypothesis, field potentials that were observed during single-unit recordings were smaller with heat stimuli than with visual stimuli.

MODULATION OF ACTION OF L-GLUTAMATE ON *APLYSIA* NEURONS

Principal Investigators: M. J. McCreery and D. O. Carpenter

At crustacean neuromuscular junction, the excitatory response of the muscle to glutamate is markedly potentiated by aspartate, which has only a small effect when applied alone. We found a similar modulation of glutamate responses by aspartate on Aplysia neurons. However, these glutamate responses are due to either chloride or potassium conductance increases rather than a sodium conductance increase as in the crustacean muscle. Most of our recordings were made from unidentified neurons in the buccal ganglion and some from the abdominal ganglion. Cells were penetrated with two independent microelectrodes for recording and current passing, respectively. Drugs were passed ionophoretically from three- or five-barrelled extracellular electrodes or from two single-barrelled electrodes whose tips were placed together microscopically. Most receptors were located in the neuropile.

On different neurons, glutamate may cause no response or specific conductance increases to sodium, chloride, or potassium, and on some cells there are biphasic responses to chloride and potassium. The sodium responses are rare, and we have not investigated those in detail. For most chloride and potassium responses, aspartate is very much less effective than glutamate and must be applied at 2-50 times the concentration of glutamate for an equal response. However, when a control ionophoretic pulse of glutamate is preceded by an ionophoretic pulse of aspartate, the glutamate response may be potentiated by as much as fivefold. As the number of preconditioning aspartate pulses is increased, the glutamate responses are first facilitated and then depressed. This suggests that aspartate can interact with and desensitize the glutamate receptor but that the modulatory action is through a different action. The potentiation is similar for chloride and potassium responses and also is not affected by the membrane potential at which the cell is tested. The potentiation is abolished in sodium-free seawater even though both the chloride and potassium responses may actually increase in size under these circumstances. In addition to causing an increase in amplitude, sodium-free seawater results in an increase in the time to peak of the glutamate response and a depression rather than facilitation when aspartate is applied with glutamate. Although cooling depresses the modulation in some experiments, in most of them the modulation is still present at temperatures as low as 5°C. Cysteate, a sulfonic acid analogue of aspartate, also causes modulation of the glutamate response and is about equally effective as aspartate. It is of interest that homocysteate, the sulfonic acid analogue of glutamate, blocks the glutamate responses and inhibits the facilitation by aspartate.

Others have ascribed the modulation of the glutamate response by aspartate, variously, to a conformational change of the receptor, inhibition of the glutamate uptake system, or induced alteration of the rate of onset and recovery of receptor desensitization. Our results are most consistent with inhibition of sodium-dependent glutamate uptake, although we cannot explain the ineffectiveness of temperature in blocking the modulation by this mechanism.

SEROTONIN CAUSES ACCUMULATION OF CYCLIC AMP IN *APLYSIA* HEART

Principal Investigators: P. R. Kebabian and D. O. Carpenter, *AFRRI*
Collaborator: J. W. Kebabian, *National Institutes of Health*

Actions of neurotransmitters have been studied in two ways. First, the voltage and conductance changes produced by substances can be characterized with electrophysiological techniques. Second, the ability of substances to produce alteration in cellular biochemistry can be characterized. However, even when both processes occur in the same tissue, it is not certain whether the electrophysiological and biochemical processes are two manifestations of one process or are totally independent. Ability of neurotransmitters to increase the rate of synthesis of cyclic adenosine-3',5'-monophosphate (cAMP) has been described in several neural tissues. However, the electrophysiological correlates of such increases have been identified in only a few tissues.

We are particularly interested in the actions of serotonin since it is one of the vasoactive and neuroactive substances released by ionizing radiation from mast cells. We describe a preparation in which serotonin acts electrophysiologically as an excitatory neuromuscular transmitter and where we find that serotonin also is a potent inducer of cAMP synthesis. This preparation is relatively simple and well studied; therefore it may be of value to study the possible interrelationship between electrophysiology and biochemistry.

These experiments used slices of the auricle or ventricle of Aplysia californica, incubated with serotonin or dopamine (30-100 μM). cAMP was measured by the method of Brown et al. (1). The ability of several drugs to interfere with transmitter-activated cAMP synthesis was determined.

In slices of Aplysia auricle or ventricle, there was stimulation of cAMP production by serotonin but not by dopamine. Incubation with serotonin caused a 16-fold increase in cAMP content of the ventricle (Figure 1) or the auricle.

We also studied the effects of several drugs on this response to serotonin. Two substances, 2-bromo-lysergic acid diethylamide (BOL) and lisuride, were very potent in blocking the serotonin-stimulated cAMP synthesis. Inhibition was essentially total at 10 μM for both substances. Fluphenazine and lergotrile also were antagonists, but they were considerably less potent and also had some agonistic actions. Metoclopramide was without effect.

We previously studied a different peripheral tissue of Aplysia: the gill (2). In this tissue, both serotonin and dopamine induce cAMP synthesis. Maximum stimulation was achieved with 30 μM dopamine and 100 μM serotonin. Since stimulation was not decreased in high-magnesium seawater (which depresses transmitter release), it was a result of direct action of the transmitters on receptors. Stimulation by serotonin and dopamine were found to be additive, but could be distinguished pharmacologically only with considerable difficulty. No drug, of over 12 studied, affected only one receptor. Only BOL and lisuride allowed any pharmacological separation of the serotonin and dopamine receptors. Both of these drugs inhibited the dopamine-stimulated cAMP production at concentrations of one order of magnitude lower than those required to depress serotonin stimulation. Present results provide very clear evidence

that the receptors that mediate serotonin and dopamine stimulation of cAMP production are distinct. Thus the heart responds to serotonin, but not dopamine, with an accumulation of cAMP; the serotonin receptor is similar to that in the gill.

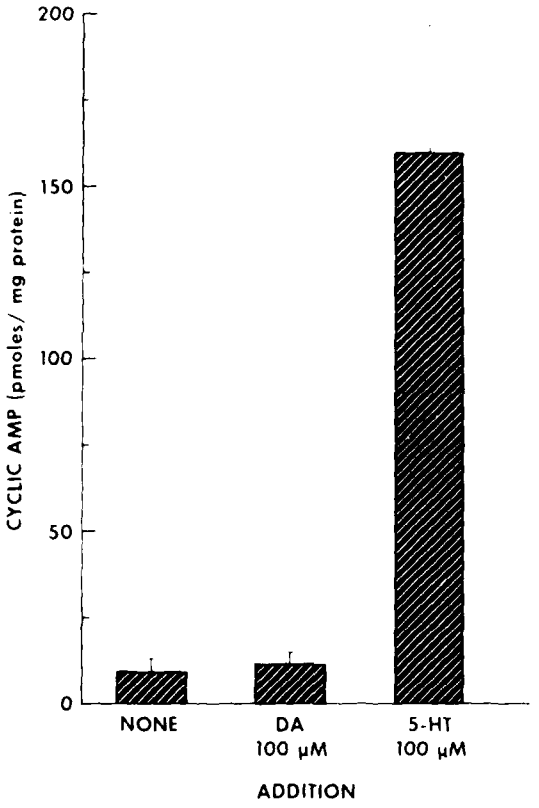

Figure 1. Effects of dopamine (DA) and serotonin (5-HT) on cyclic adenosine-3',5'-monophosphate (cAMP) content in *Aplysia* ventricle. Slices of tissue were incubated at 30°C in presence of 10 mM theophylline and neurotransmitters for 20 min. Reaction was terminated by freezing tissues on dry ice. Tissue was homogenized in 1 ml of 0.2 M hydrochloric acid in absolute ethanol. After centrifugation, homogenate was evaporated to dryness at 80°C, and cAMP content determined by method of Brown et al. (1).

Serotonin is well known to cause excitation of molluscan hearts, and evidence for an excitatory role of serotonin on Aplysia heart is strong. The heart contains and will concentrate serotonin (3), and labeled serotonin can be released on stimulation of nerves to the heart (4). A serotonin-containing neuron has been identified that makes excitatory synaptic connections to heart muscle fibers and increases the heart rate (5). With intracellular recording from heart muscle fibers, the potentials produced by this neuron have been shown to be slow depolarizations.

This preparation holds considerable potential for further study of the correlation of physiological and biochemical events associated with neurotransmitter actions. The simple electrophysiology, the homogeneous cell population, and the accessibility of

the preparation for analysis of pharmacologic sensitivities of both events may permit definitive tests of the association between cAMP synthesis and the electrical changes in muscle fibers.

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ELECTROPHYSIOLOGY AND PHARMACOLOGY OF STRIATED MUSCLE FIBERS CULTURED FROM DISSOCIATED NEONATAL RAT PINEAL GLANDS

Principal Investigators: J. E. Freschi, A. G. Parfitt, and W. Shaw

Striated muscle fibers have been observed within the pineal glands of several mammalian species, including man. We found striated muscle fibers in each of 20 consecutive pineal glands cultured from individual neonatal (2-day) rats. Subsequent experiments were done with dissociated cultures of pineal glands pooled from several litters. Myotubes were first visible after about 1 week in culture. During the next several weeks the myotubes increased in size, developed cross-striations, and began to twitch spontaneously. The resting membrane potential increased with age in culture. All myotubes studied showed delayed rectification (Figure 1). Action potentials either occurred spontaneously or could be evoked if the membrane were sufficiently polarized (Figure 2). No spontaneous endplate potentials were seen. Acetylcholine produced a brief, monophasic depolarizing response (Figure 3). Norepinephrine, serotonin, dopamine, melatonin, and γ-aminobutyric acid had no effect on the resting membrane potential when applied iontophoretically. The acetylcholine response was reversibly blocked by 10^{-6} M d-tubocurarine and irreversibly blocked by 10^{-6} M α-bungarotoxin (Figure 4). Atropine at 10^{-4} M reduced the amplitude and

shortened the time course of the acetylcholine response, and 10^{-3} M produced complete but reversible inhibition.

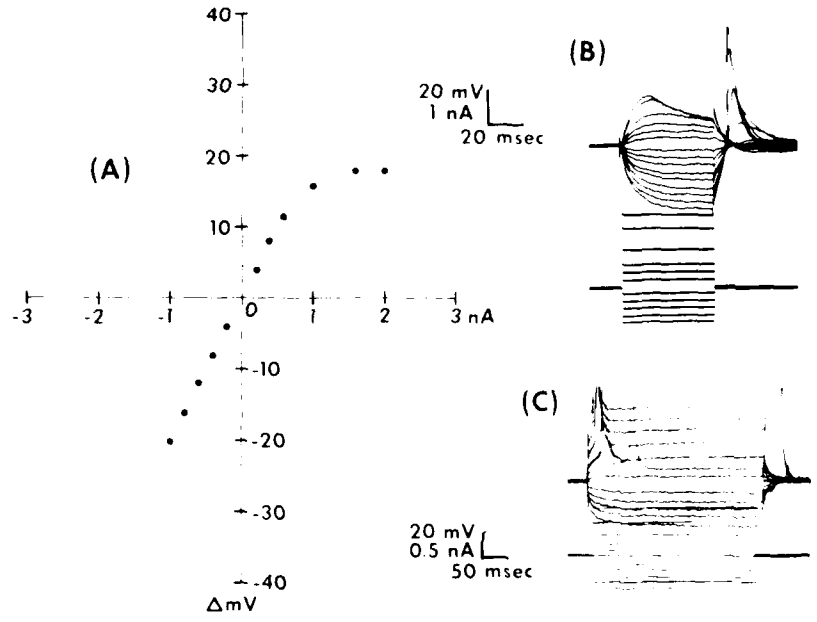

Figure 1. Current-voltage relations in pineal gland myotubes. (A) and (B) are from same myotube with resting membrane potential of -50 mV. (C) is from myotube with resting membrane potential of -60 mV. In (B), upper trace shows change in membrane potential in response to different rectangular current pulses (lower trace) of constant duration. Responses were measured at end of current pulse. In (A) these relations are displayed graphically. Origin of plot represents resting membrane potential. Note onset of delayed rectification after membrane potential is depolarized to about -35 mV. (C) Inactivation of delayed rectification during long depolarizing current pulses. Upper trace, voltage; lower trace, current.

It is concluded that myogenic cells of unknown origin occur within the neonatal rat pineal gland. These pineal muscle fibers are electrophysiologically and pharmacologically identical with peripheral skeletal muscle cells in vitro. Although the pineal gland is devoid of acetylcholine, these striated muscle fibers develop acetylcholine receptors but do not develop receptors mediating electrophysiological responses for norepinephrine, serotonin, dopamine, melatonin, or γ-aminobutyric acid, which are known to be present in the pineal. Comparison of the developmental properties of these cells in culture with other systems (such as striated muscle fibers in dissociated thymus) (1) suggests the possibility that these pineal striated muscle fibers may arise from pluripotential stem cells.

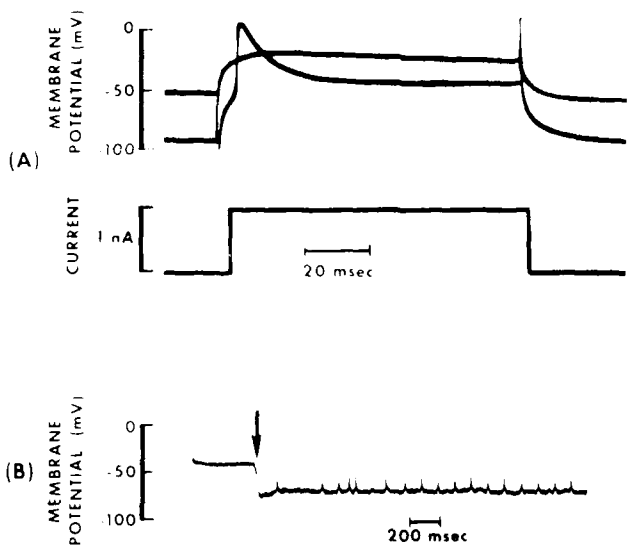

Figure 2. Dependence of action potential generation on resting membrane potential. (A) At resting membrane potential of -50 mV, current pulse evokes passive response. After hyperpolarization to -90 mV, same size current pulse elicits spike. (B) After hyperpolarization of myotube membrane from resting membrane potential -45 mV to -75 mV, spontaneous action potentials, not seen at resting membrane potential, are generated. Arrow marks onset of direct current.

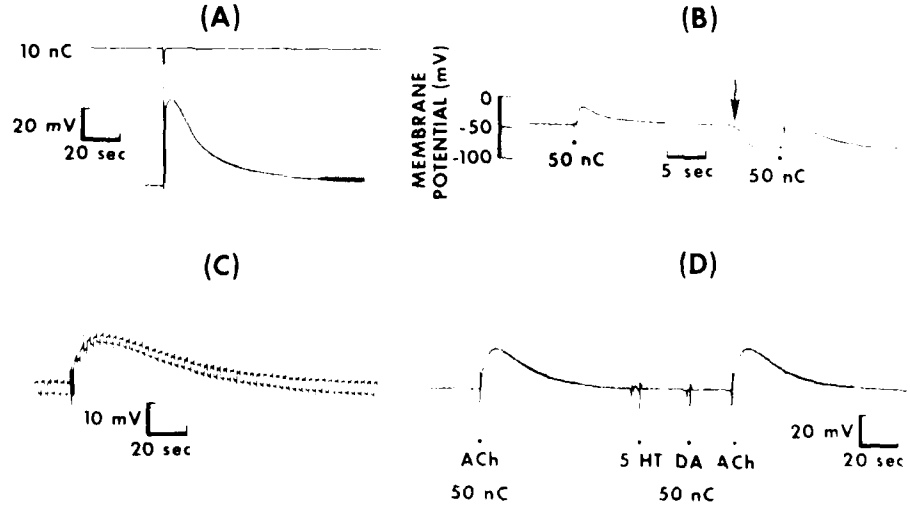

Figure 3. Depolarizing response of myotubes to acetylcholine (ACh). (A) Typical response to single pulse of ACh. Upper trace current expressed as charge, lower trace, voltage. (B) ACh depolarizing response increases in amplitude and gives rise to an action potential after membrane is hyperpolarized from resting membrane potential of -50 mV to -75 mV by passing steady inward current (indicated by arrow). Dot marks time of application of ACh. (C) Membrane resistance is monitored by passing brief, constant hyperpolarizing current pulses across cell membrane. During ACh response, resistance falls. (D) Using a multibarrelled iontophoretic pipette, +0.9 nC pulses (applied at dot) of ACh, serotonin (5HT), and dopamine (DA) are ejected at different times. Myotube responds only to ACh. Negative responses to melatonin, noradrenaline, and γ-aminobutyric acid are not shown.

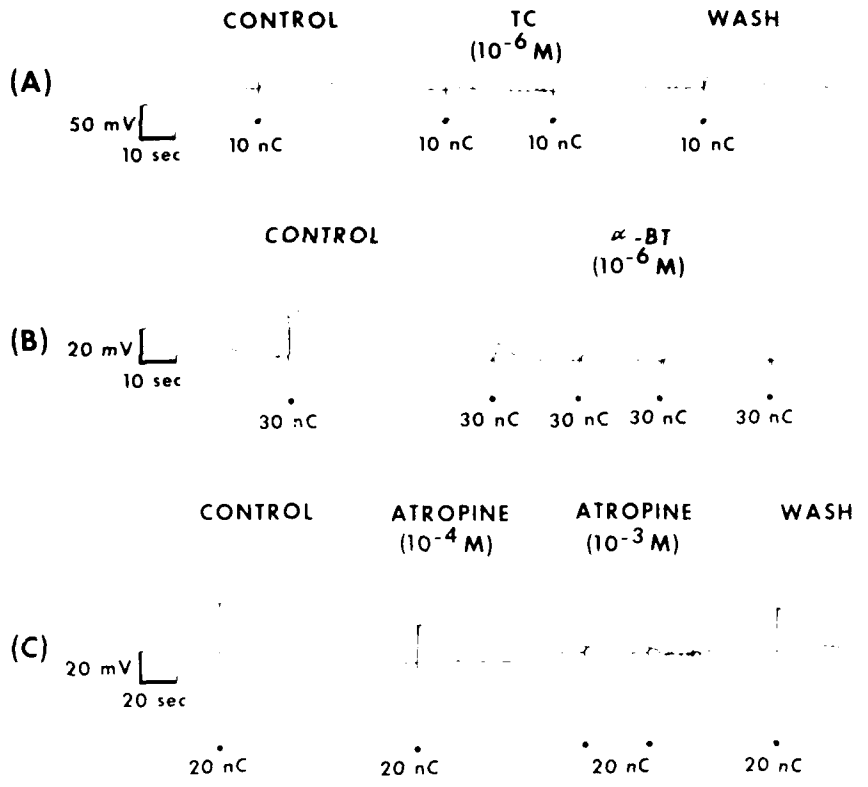

Figure 4. Effects of pharmacological antagonists on the acetylcholine (ACh) response. In all traces, dot marks time of ACh iontophoretic pulse. (A) TC (d-tubocurarine) completely blocks ACh response. Recovery of ACh response is seen after 30-min wash. (B) α-BT (α-bungarotoxin) inhibits ACh response, which is not restored after 45-min wash. (C) Atropine (10^{-4} M) reduces amplitude and shortens rate course of ACh response. Atropine (10^{-3} M) blocks ACh response. Recovery is shown after 45-min wash.

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NUCLEAR SCIENCES DEPARTMENT

The Nuclear Sciences Department is composed of two Divisions: the Nuclear Biology Division and the Radiological Physics Division.

The Nuclear Biology Division uses radionuclide counting and imaging techniques for the study of physiological functions in experimental animal models. These techniques are a unique approach to the study of radiobiological problems and medical problems of military relevance.

During Fiscal Year 1978, the Nuclear Biology Division had ten active research work units relating to five major study areas:

(a) *Pulmonary:* (i) Comparison of effects of neutron and gamma irradiation on canine pulmonary function; (ii) mathematical modeling of the uptake and clearance of radioactive gases administered via pulmonary inhalation; (iii) evaluation of methods for diagnosing pulmonary thromboembolic disease.

(b) *Cardiovascular:* (i) Development of new cardiac imaging radiopharmaceuticals based on the principle of specific receptor binding; (ii) quantitation of experimental cardiac shunts wherein surgically created left-to-right shunts are quantitated noninvasively by using radionuclide imaging techniques; (iii) evaluation of radiolabeled antibodies for assessment of cardiac function.

(c) *Radiation effects on tissue uptake of radiopharmaceuticals:* (i) Evaluation of radiation-induced soft tissue accumulation of bone-imaging radiopharmaceuticals; (ii) evaluation of effects of irradiation on biodistribution of the tumor-imaging agent gallium-67 citrate (in collaboration with Biochemistry Department, described under that program).

(d) *Bone:* Quantitative evaluation of bone repair, using a mandibular bone graft model.

(e) *Bone marrow and lymphatics:* Investigation of the usefulness of indium-111 as an agent for imaging the red bone marrow and lymphatic systems as well as its potential use in assessing radiation damage to these tissues.

The experimental models and radionuclide techniques developed and/or used in these five study areas provide the means for addressing radiobiological issues and medical problems of military significance. For example, studies on bone repair provide a useful model for the noninvasive, quantitative evaluation of traumatic or radiation-induced bone injury; the receptor-binding cardiac radiopharmaceuticals can be used for the study of effects of radiation on specific receptor sites in the heart; and the studies of radiation effects on tissue uptake of radiopharmaceuticals explore the possibility of using radiopharmaceuticals as biological dosimeters. Further use and exploration of these models are anticipated in the immediate future.

The Radiological Physics Division provides dosimetry support for all radiation sources at AFRRI. Although its function is primarily supportive in nature, it is a highly scientific function requiring extensive in-house dosimetry research. Its main areas of study are (a) measurement of tissue-to-air ratios (TARs); conversion of air doses to tissue doses using tissue-equivalent phantoms; (b) field mapping; measurement of dose distribution; (c) the study of new dosimetry systems for adaptation to the AFRRI program.

EFFECTS OF FRACTIONATED DOSES OF FAST NEUTRONS AND PHOTONS ON NORMAL CANINE LUNG: RELATIVE BIOLOGICAL EFFECTIVENESS VALUES OBTAINED BY RADIONUCLIDE STUDIES

Principal Investigators: P. O. Alderson, F. Vieras, and K. G. Mendenhall, *AFRRI*
E. W. Bradley, J. A. Deye, M. P. Fisher, and C. C. Rogers, *George Washington University*

Thirty-nine adult, male beagles received either fast neutron irradiation or photon irradiation to the right thorax. There were two nonirradiated control dogs. Twenty-four dogs received fast neutrons with a mean energy of 15 MeV to total doses of 1000, 1500, 2250, or 3375 rads delivered in four fractions per week for 6 weeks. Fifteen dogs received total doses of 3000, 4500, or 6750 rads of photons (cobalt-60) in the same fractionation pattern.

Radionuclide evaluations of pulmonary function were performed preirradiation and every 3 months postirradiation for 1 year. These included: (a) radioaerosol deposition of an insoluble radiocolloid, technetium-99m-phytate; (b) xenon-133 ventilation studies; and (c) technetium-99m-macroaggregated albumin perfusion images.

Values for the relative biological effectiveness (RBE) of fast neutrons in producing changes in these parameters have been obtained by plotting the changes from preirradiation values in the right lung as a function of the total dose. RBE values for the relative deposition of aerosol and the relative distribution of volume and perfusion have been obtained at 3, 6, 9, and 12 months postirradiation. The RBE for neutron damage to normal lung tissue was always greater than 4 in the dose range of 4000-6000 rads of photons (Figure 1).

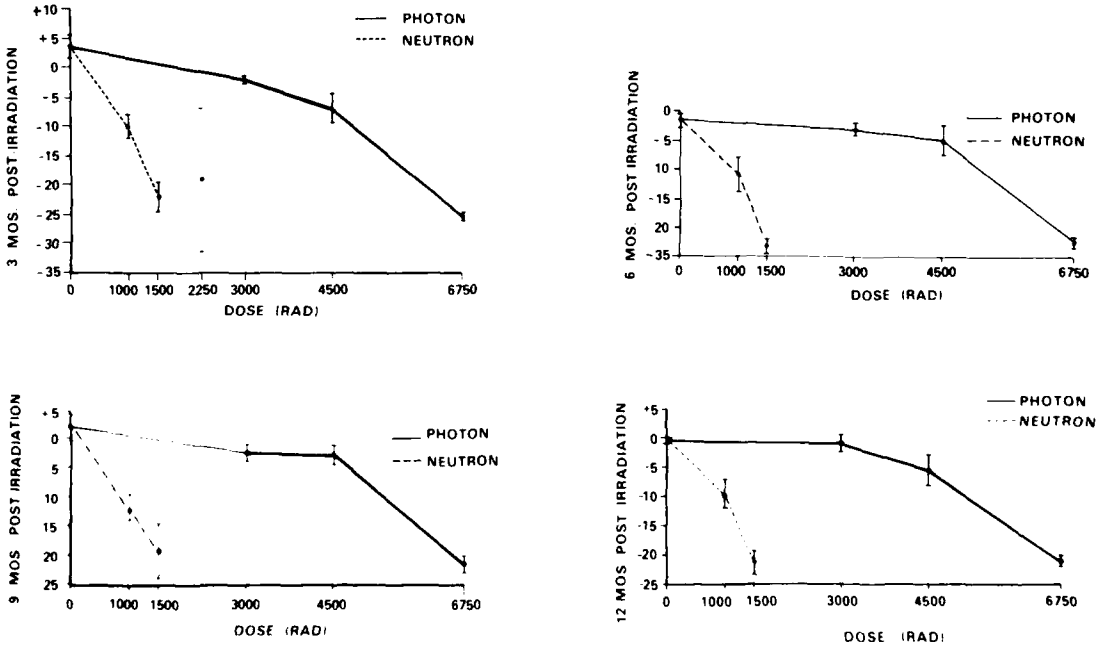


Figure 1. Mean changes (± 1 S.E.) in relative distribution of perfusion plotted as a function of dose at 3, 6, 9, and 12 months postirradiation for both photon-irradiated and neutron-irradiated dogs.

LONG-TERM RADIONUCLIDE EVALUATION OF REGIONAL PULMONARY FUNCTION AFTER IRRADIATION OF CANINE LUNG WITH COBALT-60 OR FAST NEUTRONS

Principal Investigators: F. Vieras and K. G. Mendenhall, *AFRRI*
E. W. Bradley and C. C. Rogers, *George Washington University*
P. O. Alderson, *Johns Hopkins Hospital*

Pulmonary effects of neutron and gamma radiation were compared in 31 beagle dogs subjected to hemithorax irradiation with cobalt-60 gamma rays or 15-MeV neutrons. Integral cobalt-60 doses of 3000, 4500, or 6750 rads and neutron doses of 1000, 1500, or 2250 rads were given on a therapy schedule of four equal fractions per week for 6 weeks. The dogs underwent serial technetium-99m macroaggregated human serum albumin (MAA) perfusion, technetium-99m phytate aerosol, and xenon-133 ventilation studies before irradiation and at 3, 6, 9, 12, 18, and 24 months postirradiation.

There were marked early reductions in perfusion and aerosol deposition in the neutron-irradiated dogs as well as in the high-dose gamma group. Those changes were sustained throughout the period of study in the neutron group. The high-dose gamma group showed a gradual trend toward recovery. The pattern of aerosol deposition agreed closely with the perfusion pattern and, in several dogs, the abnormalities in aerosol deposition were more prominent than corresponding abnormalities in perfusion. Abnormalities in xenon-133 clearance were minimal. The single-breath xenon-133 distribution correlated better with the aerosol deposition pattern than either the equilibrium or clearance studies. In the clinical dose range of 4000-6000 rads, the neutron relative biological effectiveness (related to decreases in lung function of equal magnitude for gamma rays) was greater than 4.

This study indicates that perfusion and radioaerosol imaging are more useful than xenon-133 studies in the long-term assessment of pulmonary radiation injury, and demonstrates that neutrons produce more persistent lung damage than do gamma rays.

QUANTITATIVE COMPARISON OF INERT GAS EXCHANGE IN DOGS

Principal Investigators: K. G. Mendenhall and F. Vieras, *AFRRI*
P. K. Weathersby, E. E. P. Barnard, and L. D. Homer, *Naval Medical Research Institute*

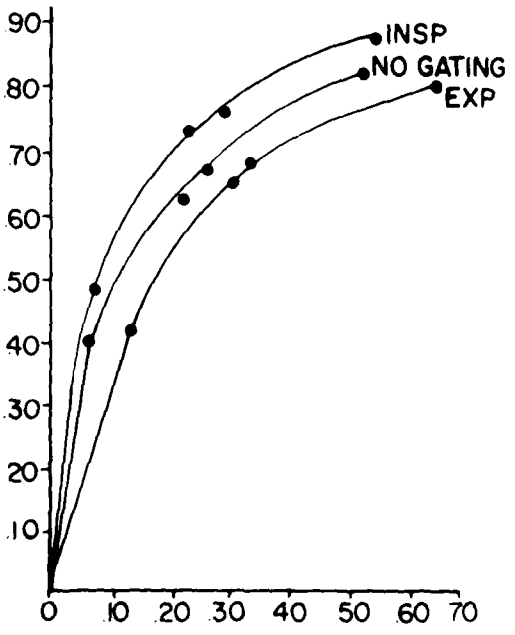
Decompression theories generally presume the existence of a number of anatomic organs or mathematical "tissue compartments" with widely varying gas exchange characteristics. In order to obtain quantitative information regarding the distribution of gas quantity and exchange rates in an animal, we allowed anesthetized dogs to breath small amounts of xenon-133 for periods of 10 to 50 min and then room air for the next 6 hours. Throughout the period, the xenon gas concentrations in large regions of the animal were measured with a gamma camera that allowed resolution of several thousand locations with dimensions on the order of 1 cm.

Exponential xenon analysis with various "half-times" did not allow useful comparisons among data sets. Instead, the results are presented in terms of moments of the residence time distribution function of each location in the dog. We obtained maps of the mean residence time (i.e., the first moment), which indicate large variations in both gas quantity and gas exchange rates within a dog. For example, in locations near the heart and in the center of the brain, mean residence times are 5 to 10 min, shaft regions of long bones have mean times of about 30 min, and some joint areas have mean residence times of over 100 min.

GATED AND CINEMATIC PERFUSION LUNG IMAGING IN DOGS WITH EXPERIMENTAL PULMONARY EMBOLISM

Principal Investigators: F. Vieras and K. G. Mendenhall, *AFRRI*
P. O. Alderson, D. F. Householder, and H. N. Wagner, *Johns Hopkins Hospital*

To determine how pulmonary respiratory motion affects detection of pulmonary emboli, 11 dogs had routine lung scans and gated or cinematic perfusion images after undergoing autologous experimental pulmonary embolism. Six dogs had routine six-view perfusion studies plus end-inspiratory and end-expiratory gated perfusion studies performed with a physiologic synchronizer set to 80% threshold. Five other dogs had three-view ungated and cinematic perfusion images (posterior, left posterior oblique, and right posterior oblique). Cinematic studies were acquired by synchronizing a camera-computer system to the Harvard respirator that ventilated the dog. Before death, all animals had received intravenous india ink to outline pulmonary perfusion defects, and postmortem lung dissection verified sites of emboli.



An ROC (receiver-operating characteristics) curve analysis (Figure 1) of randomized perfusion studies showed that end-inspiratory gated images yielded true-positive rates 5%-10% higher than ungated images at any given false-positive rate. The number of lesions detected by cinematic studies was comparable to the number detected by ungated images, but the number of lesions detected by end-expiratory images was lower. End-inspiratory gated imaging may be useful as an occasional adjunct to routine perfusion lung imaging.

Figure 1. Receiver-operating characteristics curve analysis of end-inspiratory (INSP) and end-expiratory (EXP) gated images and standard ungated images.

RECEPTOR-BINDING RADIOTRACERS: A CLASS OF POTENTIAL RADIOPHARMACEUTICALS

Principal Investigators: F. Vieras, *AFRRI*
W. C. Eckelman, R. C. Reba, R. E. Gibson, and W. J. Rzeszotarski, *George Washington University*

To date no radiopharmaceutical is routinely used to study changes in receptor concentration. Frequently, changes in receptor concentration or the appearance of receptors in tumors indicates a specific pathologic state. With a receptor-binding radiotracer, in vivo studies of these changes will be possible.

A reversible bimolecular model and in vitro tests were used to determine equilibrium constants and maximum target-to-blood ratios for new derivatives. Theoretical calculations showed that derivatives binding to the estrogen receptor, the beta-adrenoceptor, or the cholinergic receptor are capable of achieving satisfactory target-to-blood ratios.

Using in vitro tests, the apparent affinity constant was determined for five iodinated estrogen derivatives and five derivatives of beta blockers. Results of the in vitro study with derivatives of beta blockers and the in vivo displacement studies using propranolol indicated that the high heart-to-blood ratios (5:20) obtained with the new derivatives were not the result of specific interaction with the receptor. In this instance, factors other than receptor binding controlled the in vivo distribution. The in vitro assay using estrogen receptors showed that of the five derivatives, iodohexestrol and 17-alpha-iodoethynylestradiol bind to the receptor with the highest affinity. In vivo studies confirmed these results; iodohexestrol gave a uterus-to-blood ratio of 10 in immature rats when plasma-protein binding was blocked. With a tritiated muscarinic cholinergic blocking agent, heart-to-blood ratios near the theoretical maximum were obtained. This compound most closely follows the mechanism described by the model.

Use of the theoretical model in conjunction with in vitro assays can greatly aid in the design of this new class of receptor-binding radiopharmaceuticals.

DECONVOLUTION ANALYSIS IN RADIONUCLIDE QUANTITATION OF LEFT-TO-RIGHT CARDIAC SHUNTS

Principal Investigators: K. G. Mendenhall, *AFRRI*
P. O. Alderson, K. H. Douglas, and H. N. Wagner, *Johns Hopkins Hospital*
V. A. Guadiani and D. C. Watson, *National Institutes of Health*

A poor bolus injection results in an unsatisfactory quantitative radionuclide angiocardiogram in as many as 20% of children with possible left-to-right (L-R) cardiac shunts. Deconvolution analysis was applied to similar studies in experimental animals to determine if dependence on the input bolus could be minimized. Repeated good

bolus injections, prolonged injections (> 2.5 sec), or multiple-peak injections were made in four normal dogs and in seven dogs with surgically created atrial septal defects (ASD). The ratio of counts in a region of pulmonary circulation on gated acquisition to the counts in a region of system circulation (QP/QS) was determined, using the gamma function. The mean QP/QS from ten studies of good bolus injection in each animal was used as the standard for comparison.

In five trials in normal animals, when a prolonged or double-peak bolus led to a shunt calculation (QP/QS > 1.2:1.0), deconvolution resulted in QP/QS = 1.0. Deconvolution improved shunt quantitation in eight of ten trials in animals that received a prolonged bolus. Correlation between the reference QP/QS and the QP/QS calculated from uncorrected bad bolus injection studies was only 0.39 ($p > 0.20$). After deconvolution using a low-pass filter, the correlation improved significantly ($r = 0.77$, $p < 0.01$). The technique gave inconsistent results with multiple-peak bolus injections.

Deconvolution analysis in these studies is useful in preventing normals from being classified as shunts and in improving shunt quantitation after a prolonged bolus. Clinical testing of this technique in children with suspected L-R shunts seems warranted.

STUDY OF HOST-GRAFT ACTIVITY PATTERNS IN DOGS WITH MANDIBULAR BONE GRAFTS USING QUANTITATIVE BONE IMAGING

Principal Investigators: F. Vieras and K. G. Mendenhall, *AFRRI*
R. G. Triplett and J. F. Kelly, *Naval Medical Research Institute*

We have investigated the use of quantitative radionuclide bone imaging for assessment of healing in mandibular bone grafts. Full-thickness mandibular defects were surgically created in beagle dogs and replaced by either allogeneic grafts (homografts) or xenogeneic grafts (heterografts). Allografts (from allogeneic freeze-dried mandibles) provided a model of successful grafts whereas xenografts (from sheep mandibles) provided a model of graft failure.

Grafts were evaluated by (a) radionuclide imaging with technetium-99m stannous diphosphonate using a gamma camera-computer system, (b) radiography, and (c) clinical examination, all performed at 1, 2, 4, 6, and 8 weeks postgraft. Activity in the graft and proximal host bone was expressed as a ratio of counts per element in the region of interest to counts per element in a control region in the contralateral side of the mandible.

In the allografts ($n = 5$), the serial mean activity ratios gradually approached those of the host area. In the xenografts ($n = 4$), the mean activity ratios never reached the level of the host (Figure 1). Healing occurred in all animals in which the activity ratios in the graft approached or were greater than the host by 6 weeks postgraft whereas the grafts with ratios remaining considerably below those of the host did not heal. Radiographic evaluation during the 8-week period of study was not a reliable indicator of graft success or failure. Radionuclide bone imaging appears useful for the early identification of failure or success of a graft.

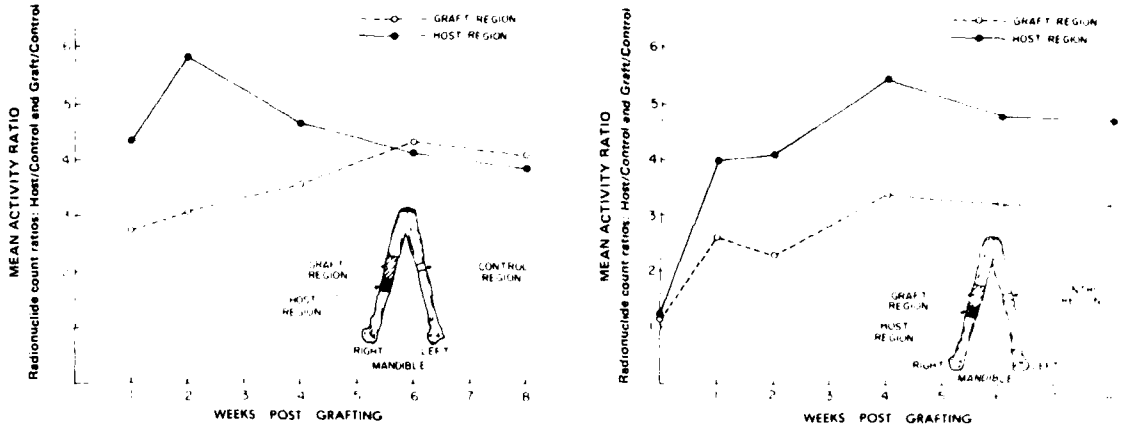


Figure 14. Comparison of mean activity in allogeneic bone grafts and proximal host bone. *B*. Comparison of mean activity in xenogeneic graft and proximal host bone

EVALUATION OF INDIUM-111 COLLOID FOR ABDOMINAL LYMPH NODE IMAGING

Principal Investigators: F. Vieras and M. P. Grissom

During investigations of the usefulness of indium-111 chloride for red bone marrow imaging, indium-111 was noted to show a strong tendency to form a colloid of small particle size at basic pH. This observation prompted evaluation of an indium-111 colloid for radionuclide imaging of the lymph nodes.

The procedure for preparation of indium-111 colloid is simple and yields a labeling efficiency of over 99%. The particle size, the most important parameter influencing the biologic behavior of radiocolloids, was found to be very small ($< 0.1\ \mu m$). Excellent abdominal lymph node images were obtained in experimental animals after subcutaneous injection in the feet (Table 1).

Indium-111 colloid is an excellent choice of radiopharmaceutical for noninvasive evaluation of the abdominal lymph nodes.

Table 1. Radiation Dose Estimated for 1.0 mCi of Indium-111 Colloid Injected Subcutaneously in Feet

Organ	Dose (Rads)
Liver	0.916
Spleen	0.494
Bone Marrow	0.006
Lower Trunk	4.480
Upper Legs	4.670
Feet	5.690
Total Body	0.563

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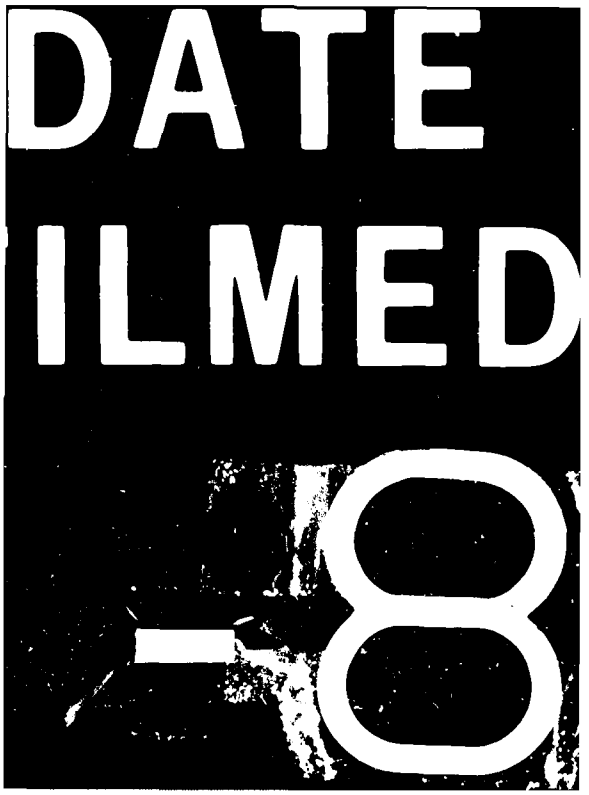
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